THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 105 AUGUST 1, 1933 No. 2

THE DETERMINATION OF THE POTENCY OF BEE VENOM *IN VITRO*

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Received for publication May 25, 1933

At one point in a study of reactions provoked by the injection of bee venom into an animal system it seemed desirable to evolve a more rapid method than the test *in vivo* for determining the potency of the venom, which required two weeks for a complete reading of the data.

The venom of the honey-bee, *Apis mellifica*, was used throughout this study. The venom was titrated and its effects *in vitro* on blood corpuscles and *Paramecium caudatum* have been compared with its effects on guinea pigs.

METHODS. Venom collected by various methods exhibited no marked differences in the reactions it produced. In some cases it was dried in the dissected stinging apparatus; in others it was used fresh from the tip of the sting or from ruptured poison glands. The following method, as a standard, was finally adopted after animal tests had shown that, by its use, the typical qualities of the venom had been preserved.

The stings and their attached glands were carefully teased out of the abdomens of anesthetized bees. The entire poison apparatus was dried quickly *in vacuo* over anhydrous phosphorus-pentoxide and sealed in glass tubes until used.

For a potency test the dried apparatus of one bee was added to 1 cc. of sterile tap water (or physiological salt solution if the mixture were subsequently to be injected) in an agglutination tube. Since a single bee secretes about 0.0003 gram of venom (1) this original dilution was approximately 1 part of venom in 3000 parts of water. The resulting solution was thoroughly mixed and shaken. Five-tenths cubic centimeter were removed from this original dilution and added to 0.5 cc. of sterile tap water in a second tube of the series. In order to prevent macerated bits of tissue from being carried over from the original dilution, the liquid was drawn into the

pipette through a small piece of cotton. The contents of the second tube were likewise shaken and the process was repeated through ten more dilutions.

Cytolytic reactions to Paramecia. An attempt was made to employ pure cultures of *Paramecium caudatum* (2) as a rapid indicator of the strength of the venom in a known dilution.

Fifteen to 20 Paramecia, taken from 5-day potato-water cultures in about 0.01 cc. of their culture media, were added to the contents of each agglutination tube in the series of dilutions and the effects of the venom were observed and timed.

TABLE 1

Time for cytolysis of paramecium

BEE STING NUMBER	DILUTION					
	I*	II	III	IV	V	VI
	1:3,000	1:6,000	1:12,000	1:24,000	1:48,000	1:96,000
		seconds	*seconds*	*minutes*	*minutes*	
1	Immediately	10	20	2	60+ Few dead	−†
2	Immediately	5	30	2	60+ Few dead	−
3	Immediately	5	20	3	60+ Few dead	−
4	Immediately	10	20	3	60+ Few dead	−
5	Immediately	5	35	3	−†	−
6	Immediately	5	20	4	−	−
7	Immediately	5	20	5	−	−
8	Immediately	7	25	5	−	−
9	Immediately	5	20	5	−	−
10	Immediately	10	20	13	−	−

* This dilution approximates the unit weight of venom originally used by Langer.
† No cytolysis took place within the two-hour limit.

Death of the protozoa from complete cytolysis resulted in dilutions 1 to 5, the time required depending on the potency of the venom in the solution. In the higher dilutions where cytolysis could be observed, it was preceded by a rapid but gradually diminishing spinning of the Paramecia. The long slipper form gradually swelled, developed irregular processes, then ruptured and released the cell granules into the surrounding liquid.

The results of a series of titrations of 10 bee stings are shown in table 1. In those dilutions of the venom where the protozoa lived, they were observed for 48 hours, during which time they apparently carried on their normal functions. Since no reactions occurred after tube 6, tubes 7 to 12 were not included in the table.

Pathological reactions on guinea pigs. To make a comparison between the effects of the various concentrations of the venom on Paramecia and on guinea pigs, a series of the same dilutions which had been lethal to the protozoa through the fourth dilution, partially lethal in the fifth and non-lethal in the sixth dilution, was injected intradermally into a guinea pig at six different sites along the right and left flanks.

The titration of the venom was made in a weak saline solution (0.425 per cent) that was innocuous to the protozoa and yet prevented the masked result which would have occurred had an aqueous solution of the venom been introduced into the skin. Water injected intradermally into a guinea pig produces a marked reaction and lesion.

The contents of tube 6 were injected first at a posterior site on the left flank. The subsequent injections progressed in order on alternate flanks toward the head.

The results of the injections were as follows:

The immediate reactions resulting from the injection of the lowest dilution were not unlike those produced by the sting of the bee. A reddening and edema directly followed the injection and provoked the guinea pig to scratch and bite at the site. The following day a necrosis was evident which embraced an area 6 mm. by 3 mm. This area persisted until the 6th day, at which time the dried necrotic tissue sloughed away leaving an open crater surrounded by a rim of granulation tissue. By the 13th day the crater had closed leaving but a hard papule at the site of injection. This papule persisted in diminishing intensity until on the 16th day no trace of the lesion remained.

The second dilution likewise produced a necrotic lesion, but it was much less intense and embraced an area 3 mm. by 2 mm. This was surrounded by a red flare that extended about 10 mm. from the necrotic patch. The small necrotic patch dried, lifted about the edges and sloughed on the 6th day after injection leaving a small open crater surrounded by a narrow rim of granulation tissue. The crater, 2 mm. across, had completely healed by the 11th day and a papule at the site of injection persisted until on the 15th day no trace of the lesion remained.

The third dilution produced a soft swollen area, the day after injection, which was 8 mm. by 5 mm. Necrosis did not develop in this wound. A thin superficial scab about 5 mm. in diameter formed about the site of injection. This was shed on the 6th day after the injection and left a hard papule with a diameter of 4 mm. This papule persisted in diminishing intensity until the 13th day when no trace of the lesion remained.

The fourth, fifth and sixth dilutions merely produced papules of varying intensity at the sites of injection. The papule produced by the fourth dilution embraced an area with a diameter of 6 mm. the first day after injection which decreased in intensity until the 13th day when no trace of the lesion remained. The papule produced by the fifth injection was less pronounced than that of dilution 4 and embraced a slightly raised area 5 mm. in diameter which completely disappeared by the 7th day. The papule produced by dilution 6 was slightly more than a red spot on the skin. There was a slight edema on the day following the injection but no trace of it remained after the 3rd day. A red spot about the site of injection persisted until the 6th day.

TABLE 2

Time of hemolytic reaction

BEE STING NUMBER	DILUTION					
	I*	II	III	IV	V	VI
	1:3,000	1:6,000	1:12,000	1:24,000	1:48,000	1:96,000
Two per cent suspension of horse corpuscles						
	seconds	*seconds*	*minutes*	*minutes*	*minutes*	*minutes*
1	5	20	3	6	36	−†
2	5	20	3	11	27	−
3	5	35	3	7	27	60+
4	6	35	7	20	−†	−
5	6	50	8	15	60+	−
6	5	55	4	20	−	−
7	5	60	4	13	34	−
8	6	60	8	16	−	−
9	7	60	4	13	34	60+
10	7	70	4	9	27	60+
Two per cent suspension of rabbit corpuscles						
11	12	35	5	14	−†	−
12	15	30	3	14	60+	−
13	15	35	5	16	−	−
14	17	35	4	11	−	−
15	20	35	5	8	60+	−
16	15	45	4	9	31	−
17	20	45	7	20	−	−
18	15	60	7	9	60	−
19	15	60	6	11	60	−
20	20	60	8	19	−	−
Two per cent suspension of guinea-pig corpuscles						
21	8	15	3	12	60+	60+
22	10	17	3	5	60+	60+
23	8	20	3	9	33	−
24	8	30	3	11	60+	60+
25	9	27	3	7	60+	−
26	7	30	6	17	40	−
27	9	30	5	19	40	−
28	10	35	6	20	32	−
29	6	60	2	7	52	−
30	6	60	3	9	−†	−

* This dilution approximates the unit weight of venom originally used by Langer.
† No hemolysis took place within the two-hour limit.

These data indicate that, like the cytolytic test, the reaction provoked by the venom reaches an end point in the fifth dilution. Definite necrosis is evident in the two lowest dilutions. A papular lesion and a superficial scabbing resulted from the third dilution. In the last three dilutions papules alone, of decreasing intensity, arise until the lesions resulting from the injections of the fifth and sixth dilutions are little more than needle punctures.

Hemolytic reactions. The hemolytic effect of bee venom has long been recognized. It was observed by Langer (3), Morgenroth and Carpi (4), Murakami (5), and Essex and his co-workers (6). From the work of these authors, it seemed that the hemolytic phenomenon might be employed as an indicator of the potency of the venom in a given dilution.

For these tests the poison from one bee was dissolved in 1 cc. of physiological salt solution and from this the subsequent dilutions in a series were made. Five-tenths cubic centimeter of a 2 per cent suspension of washed blood cells was added to a given dilution of the venom and the time for the completion of hemolysis was recorded. When the time for hemolysis exceeded a few seconds, the tubes were kept in a water bath at 37°C.

Table 2 presents data for three series of ten tests each made for comparative purposes on a 2 per cent suspension of washed blood cells from a horse, rabbit, and guinea pig.

From the tabulated results, variance will be observed in the reactions produced that indicates a slight difference from bee to bee. There is also a variance in the effect of the venom on the three groups of blood tested. However, the ten tests recorded for each blood sample on table 2 are not sufficient to demonstrate clearly this variance between the blood groups.

In their entirety, however, the results were comparable. Hemolysis in the lowest dilution took place within 20 seconds. In the second dilution it occurred in about a minute; in the third dilution within 10 minutes and within 20 minutes in the fourth dilution. In the fifth dilution hemolysis in some cases occurred in about ½ hour; in others it did not take place within the two-hour limit for the tests. This dilution appeared to be the end point for definite reaction. Hemolysis rarely occurred in the sixth dilution within the time limit; a negative sign on the table denotes this extreme. As in the cytolytic and intradermal tests, these show an end point of reaction at approximately the same dilution of venom.

CONCLUSIONS

Cytolytic and hemolytic tests made *in vitro* evince results whereby it is possible, in a short time, to determine the potency of a known dilution of bee venom. The results of these tests *in vitro* were comparable to those obtained by injecting intradermally the same dilution of bee venom into a guinea pig. This latter method for determining the potency of the venom

required about two weeks for a complete reading of the data, whereas the former tests were complete within two hours.

The tests *in vitro* showed clearly the variance in reactions provoked by the venom of individual bees. The hemolytic determinations demonstrated further that there is a slight variance in the effects of the venom on the blood cells of different kinds of laboratory animals employed. The data recorded in table 2, however are not sufficient to show the significance of this variance.

REFERENCES

(1) LANGER, J. Arch. exp. Path. u. Pharm., 1897, xxxviii, 386.
(2) GLÄSER, R. W. AND N. A. CORIA. Journ. Exp. Med., 1930, li, 787.
(3) LANGER, J. Arch. internat. pharmacod. et de thérap., 1899, vi, 181.
(4) MORGENROTH, J. AND U. CARPI. Berl. klin. Wochenschr., 1906, no. 44, 1424.
(5) MURAKAMI, K. Okayama Igakkai Zasshi, 1928, xl, 760.
(6) ESSEX, H. E., J. MARKOWITZ AND F. C. MANN. This Journal, 1930, xciv, 209.

THE INFLUENCE OF THE VAGUS NERVE ON THE GLYCEMIC LEVEL

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Received for publication May 24, 1933

Clark (1926, 1927 and 1931) has assembled evidence to indicate that the right vagus nerve contains fibers which exert an inhibitory action on the islet tissue in the pancreas. The presence of secretory fibers in the vagus seems to be on a fairly sound foundation (see deCorral, 1918; McCormick, Macleod and O'Brien, 1923; Britton, 1925; and LaBarre, 1927). The work of Hédon and Giraud (1920), Gayet and Guillaumie (1927) and Wulf (1929) among others indicates, however, that vagus impulses are not necessary for the maintenance of the normal blood-sugar level. Since Clark's results have not been reviewed, it seemed of interest to test again the evidence of inhibitory vagal fibers.

METHODS. Because of the well-known influence of anesthetics on carbohydrate metabolism it was decided to devise experiments not requiring their use. Cannon, McIver and Bliss (1924) and Britton (1928) have emphasized the influence of sympathico-adrenal activity on the glycemic level. In addition to normal cats, therefore, several which had been sympathectomized were used in these experiments. Any suffering from malnutrition or infection were avoided. All animals were kept in the laboratory one week before the control sugar tolerance was determined; during that time they were well fed on the regular laboratory diet which included liberal supplies of vitamins A, B and D.

Cats were chosen, since on them complete sympathectomy is readily accomplished. The operation was performed according to the method described by Cannon, Newton, Bright, Menkin and Moore (1929). Failure of the hairs to rise when the animal was exposed to cold or to a barking dog was the criterion used for testing the completeness of the sympathectomy. In addition, a careful autopsy, performed upon each animal, confirmed the operation.

Before the experiments the animals fasted for 18 to 24 hours. To obtain blood, the ear was shaved, the marginal vein nicked, and 0.1 cc. was drawn into the standard pipette. Two blood-sugar determinations (Folin

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and Svedberg, 1930) were made, at least 20 minutes apart, for controls. Thereupon a solution of 20 per cent dextrose (Merck) was injected intracardially; the animals had been previously trained to lie quietly on the animal board during this procedure. The dosage was 0.9 to 1.0 gram dextrose per 1.0 kgm. body weight. In general the larger cats were given the former and the small the latter dosage. In all cases the body weight did not vary in the individual more than 0.2 kgm. in the two observations before and after vagal section, and hence the actual dosage is not a factor to be considered in these experiments. The dextrose solution was made up fresh every day with distilled water. It was warmed to body temperature before injection. A number 18 needle was used. There were two fatalities with the method in more than 60 injections, one from puncture of a coronary artery and the other from puncture of the left auricle.

One blood-sugar determination was made 20 minutes after the injection of dextrose, and three or four others at 30-minute intervals. In the first experiments a test of the blood sugar was made every 20 minutes, but it was soon noted that the sugar curve always fell in practically a straight line, and for economy the interval was lengthened.

After a control sugar tolerance had been determined one vagus nerve was cut in the neck. A week later, when the animal had completely recovered and its body weight had returned to the previous figure, the sugar tolerance was again determined. Observations similar to those represented in figure 1 were made on 6 normal cats with right vagus section, on 4 sympathectomized cats with right vagus section, and on 3 sympathectomized with left vagus section. In addition, control observations were made on 14 other animals. In the latter group were included 2 that died at the time of the second tolerance test mentioned above, and 12 that developed feline distemper and were considered unfit for further study. In several instances the tests were repeated after three or four days to check on the accuracy of the method. Two animals, observed more than two weeks after the vagus section, were etherized, and the cut vagus, after being cut again above the first section, was stimulated peripherally in order to learn by observing the heart rate whether the fibers had regenerated. The results were negative.

Results. The results of three typical experiments are given in figure 1. With these is included a copy of figure 1 of Clark's paper (1931), which depicts the averages of three experiments on cats. The variations in the height of the curves in the different animals is partially due to the differences in dosage. It is interesting to notice that the glycemic rise in no. 848, an unanesthetized, sympathectomized cat before and after right vagus section, is very nearly twice the rise in Clark's experiments on anesthetized animals with the same dosage. The explanation of this difference is not clear at present. The graphs of no. 1028 and no. 1045 are, respectively,

from observations on a sympathectomized animal before and after left vagus section and on a normal before and after right vagus section.

Inspection of figure 1 reveals that in the three animals mentioned the response is practically identical before and after sectioning either the right or the left vagus. The differences between the results before and after vagus section are well within the experimental error of the method. Cat 1045 gave the least increase of all the animals studied. In this type of

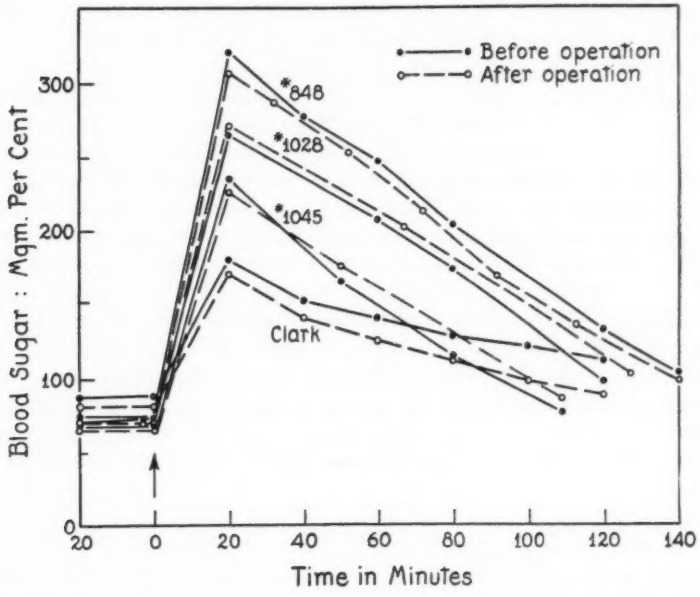

Fig. 1. Clark—The averages of 6 experiments in cats, 3 before and 3 after right vagus section; 1 gram dextrose per 1 kgm. body weight was injected at 0 minute (Journ. Physiol., 1931, lxxiii, 297).

No. 848—26-i-'32	3.9 kgm.	Sympathectomy completed one month previously. Experiment before operation.
27-i-'32		Right vagus cut in neck.
2-ii-'32	3.9 kgm.	Experiment after operation.
No. 1028—19-v-'32	3.5 kgm.	Sympathectomy completed 3 weeks previously. Experiment before operation.
20-v-'32		Left vagus cut in neck.
25-v-'32	3.3 kgm.	Experiment after operation.
No. 1045—30-iii-'32	3.85 kgm.	Experiment before operation.
7-iv-'32		Right vagus cut in neck.
12-iv-'32	3.85 kgm.	Experiment after operation.

In cats 1028 and 1045 the amount of dextrose injected in each experiment was 0.9 gram per kgm.; cat 848, 1.0 gram per kgm. Dextrose injected at 0 minute.

experiment, however, in which the figures are compared on the same unanesthetized cat before and about one week after the operative procedure, more reliable conclusions can be reached than in such experiments as Clark's, done under anesthesia on different animals which have been subjected to acute operation two hours before the tests. The effect of variations in the depth of anesthesia over the course of several hours in Clark's experiments may be responsible in part for the differences between the glycemic levels in these and in his results. It is perhaps significant in this connection that if the blood-sugar values in Clark's figures for the animals with the right vagus cut are increased by only 12 mgm. per cent, they will be found to coincide, practically, with the figures of his normals.

The influence on the glycemic level of medulli-adrenal secretion evoked by emotion was considered negligible in these experiments. When a normal cat was excited for 2 minutes, Britton (1928) found that the glycemic level would be increased 26 mgm. per cent on the average, while Cannon, Newton, Bright, Menkin and Moore (1929) found an average increase of 34 mgm. per cent, and in sympathectomized cats no consistent increase. In connection with this problem the glycemic level was observed in several normal cats before and after they were caged and exposed to a barking dog for one minute. The maximum increase noted was 20 mgm. per cent, and the time required for the return to the previous level varied from 60 to 120 minutes. In sympathectomized animals emotional glycemia was not an operating factor, and hence the results in this group of experiments are especially reliable. Among normal animals care was taken to select such as were placid, and then, as indicated above, they were trained to lie quietly.

SUMMARY

Study of the glycemic level in unanesthetized normal and sympathectomized cats, which have received dextrose before and after section of the right or left vagus nerve, fails to reveal any differences in the blood-sugar curves (see fig. 1) which would indicate the presence of inhibitory fibers to the islets of Langerhans in either nerve (cf. Clark, 1931).

REFERENCES

Britton, S. W. 1925. This Journal, lxxiv, 291.
1928. Ibid., lxxxvi, 340.
Cannon, W. B., M. A. McIver and S. W. Bliss. 1924. Ibid., lxix, 46.
Cannon, W. B., H. F. Newton, E. M. Bright, V. Menkin and R. M. Moore. 1929. Ibid., lxxxix, 84.
Clark, G. A. 1926. Journ. Physiol., lxi, 576.
1927. Ibid., lxiv, 229.
1931. Ibid., lxxiii, 297.
deCorral, J. M. 1918. Zeitschr. f. Biol., lxviii, 395.

FOLIN, O. AND A. SVEDBERG. 1930. Journ. Biol. Chem., lxxxviii, 85.
GAYET, R. AND M. GUILLAUMIE. 1927. Compt. rend. Soc. Biol., xcvii, 1613.
HÉDON, E. AND G. GIRAUD. 1920. Ibid., lxxxiii, 330.
LABARRE, J. 1927. Ibid., xcvi, 193.
MCCORMICK, N. A., J. J. R. MACLEOD AND M. K. O'BRIEN. 1923. Trans. Roy. Soc. Canada, Sec. V, xvii, 57.
WULF, H. 1929. Biochem. Zeitschr., ccxiv, 382.

THE EFFECT OF ADDING LIVER TO THE DIET UPON THE GROWTH AND REPRODUCTION OF RATS

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Received for publication December 2, 1932

It has been demonstrated by investigators in invertebrate nutrition (1), nutrition of fish (2), and mammalian nutritional studies (3), (4), that the addition of raw liver to a diet containing all of the known vitamins stimulates growth and general well-being.

The object of the present experiment was to determine the effects of adding liver to a stock diet, various milk diets, and a standard basal ration. The animals used for this work were a mixture of albinos and hooded rats.

In experiment I, rats having an average age of one month were placed on a diet of whole grain biscuit, fresh milk and fresh vegetables—diet 201. Three experimental groups received the following diets: group 1; diet 201 only, 5 rats, 3 males, 2 females. Group 2; diet 201 + 2 grams raw liver per rat daily, 8 rats, 2 males, 6 females. Group 3; diet 201 + 2 grams autoclaved liver (15 lbs., 120°C., ½ hr.) per rat daily, 8 rats, 5 males, 3 females. The males of group 2 averaged 60 grams heavier at the end of 9 weeks than the male rats of group 1. The males of group 3 averaged 44 grams heavier than the males fed only diet 201. Female rats which received raw liver averaged 9 grams heavier and those which received autoclaved liver averaged 19 grams heavier than the females on diet 201.

Experiment II demonstrates the beneficial effect of adding small amounts of raw or heated liver to exclusive milk diets. The rats used in these experiments averaged approximately one month of age, and the duration of the experiment was 70 days. All of the milk diets had 0.5 mgm. Cu (as $CuSo_4$), 1 mgm. Fe (as $FeCl_3$) and 0.1 mgm. I (as KI) added to each 100 grams of milk (5). The milk was brought quickly to the boiling point in an open kettle, and the liver was boiled for 5 minutes. The following diets and numbers of animals per diet were used: 202, heated milk + 5 grams raw liver per rat daily, 5 rats; 203, heated milk + 5 grams heated liver per rat daily, 5 rats; 204, heated milk only, 3 rats; 205, Recolac,[1] diluted 1 part to 7 water, + 5 grams raw liver per rat daily, 5 rats; 206, Recolac, same

[1] Recolac, a commercial dry-milk baby formula manufactured and kindly supplied by Mead Johnson Company. We also wish to thank Mead Johnson Company for cod liver oil, wheat germ oil and Viosterol.

dilution, 5 rats; 207, evaporated milk, diluted 1 part to 1 water, 5 rats; 208, evaporated milk, same dilution, + 5 grams raw liver per rat daily, 5 rats; 209, evaporated milk undiluted, 5 rats; 210, heated milk + 5 per cent lemon juice, 4 rats; 211, heated milk + 5 per cent lemon juice + 5 grams raw liver per rat—daily, 5 rats. Upon these diets the rats exhibited the following average weight gains at the end of 70 days: 202, 125 grams; 203, 149 grams; 204, 111 grams; 205, 119 grams; 206, 112 grams; 207, 108 grams; 208, 128 grams; 209, 87 grams; 210, 104 grams; 211, 138 grams. All diets have been benefited by the addition of liver. Lemon juice is often added to milk in the diet of infants, to increase the digestibility of the curd and also to give added vitamin C. However, lemon juice seemed to be of no benefit to rats.

In the next series of experiments the effect upon growth of the addition of small quantities of raw liver to a basal casein-dextrin diet (diet 212) was tested. The basal diet consisted of the following ingredients: unpurified casein 18 per cent, McCollum's salt mixture no. 185, 4 per cent, yeast 8 per cent, NaCl 1 per cent, bleached dextrin 69 per cent. Three cubic centimeters of viosterol were thoroughly mixed into each kilogram of dry food. Four per cent of cod liver oil and 2 per cent of wheat germ oil were added to each daily feeding. In addition to the basal diet, certain rats also received 5 grams raw liver or liver boiled for 5 minutes. Three groups of 5 rats each, 2 males and 3 females were used, one group receiving only diet 212, the second diet 212 with the addition of 5 grams raw liver per rat daily, and the third group diet 212 and 5 grams heated liver per rat daily. The liver addition had a decidedly beneficial effect upon the growth of the animals, the average gain in weight being 51 grams greater when raw or heated liver supplemented diet 212.

No young were born to the three females which received only diet 212, while two of the females from the raw liver group had litters of 9 and 12 young each, and one from the heated liver group had a litter of 10 young. The individuals of the litter which had 12 young averaged 71 grams in weight at the age of 1 month, those from the litter of 9 averaged 61 grams and those from the litter of 10, 74 grams. As the stock young usually average about 45 grams at the age of 1 month, these young can be considered much above average weight.

A second experiment was done using diet 212. This was continued for 3 months instead of 9 weeks, the duration of the first experiment in which diet 212 was used. Four male and 7 female rats received only diet 212 while 3 male and 9 female rats received diet 212 and 5 grams raw beef liver daily. In this experiment also, the addition of liver to the diet greatly improved the growth of the animals. At the conclusion of the 3 month period, the rats which received raw liver averaged 60 grams heavier than the similar animals which received no liver.

In reproduction the group given liver was also superior. A total of 7 litters was born to the rats which received only diet 212, but only 3 females reared their litters. Of the 19 young from these 3 litters, 16 were weaned. Five litters were born to the rats which received liver and no female failed to rear her young. Of the 47 young born, 36 were weaned. (The high mortality in both groups was doubtless due to the screen-bottomed cages, even though the females were caged separately and given shredded paper for nests.) The number of young per female in the liver group averaged 7.2, but the average weaning weight was slightly greater than among the young from the females which received no liver, average number of young per litter 5.3. The young from the group which received liver averaged at one month of age 49 grams for females and 52 grams for males. In the group which was fed only diet 212, the female young averaged 46 grams and the males 43 grams, at the age of one month.

Discussion. In all of these experiments, the addition of raw or heated liver to the diet had a beneficial effect upon the growth of the animals.

Hitchcock (6) demonstrated the value of adding rare beef to a well-balanced stock diet, to which was also added raw cabbage or lettuce once per week. In these experiments also, in which the liberal stock diet was supplemented by daily additions of fresh milk and vegetables, raw or autoclaved liver greatly benefited the growth of the animals.

As the rats fed the milk diets were each consuming from 50 to 75 cc. of milk at the close of the experiment, the value of the liver could not have been due to increasing any of the known vitamins.

That fresh liver has a beneficial effect upon growth when added to a purified ration containing all of the known vitamins has been demonstrated by several investigators. These experiments demonstrate that raw liver stimulates growth and probably also lactation, because when it was added to the diet more young were reared by the rats. Mapson (7) stated that raw liver stimulated lactation, and Daggs showed (8) that lactation was improved in dogs when raw liver was the source of protein.

SUMMARY

1. Rats were fed upon an adequate stock diet, consisting of mixed grains, fresh milk and vegetables, to which raw or heated beef liver was added. These animals showed a superior growth to those which received no liver.

2. Five grams of raw liver per rat daily added to milk diets produced superior growth in the animals over the unsupplemented milk diets, and this was true whether the milk was fresh, evaporated, or a powdered milk formula used for babies (Recolac).

3. When 5 grams of raw liver per rat daily supplemented a basal casein-dextrin diet, containing all of the known vitamins except vitamin C, the value of the diet in growth-promoting power was greatly enhanced. The

number of young reared by the rats was greater and the young were heavier at 30 days of age.

REFERENCES

(1) WULZEN, R. Univ. Calif. Pub. Physiol., 1926, vii, 1.
(2) MCCAY, C. M. Trans. Amer. Fish. Soc., 1928.
(3) OSBORNE, T. B. AND L. B. MENDEL. Journ. Biol. Chem., 1926, lxix, 661.
(4) EVANS, H. M. AND G. O. BURR. Proc. Soc. Exp. Biol. and Med., 1927, xxiv, 740.
(5) WADDELL, J., H. STEENBOCK AND E. B. HART. Journ. Nutrition, 1931, iv, 53.
(6) HITCHCOCK, F. A. This Journal, 1926, lxxix, 218.
(7) MAPSON, L. W. Biochem. Journ., 1932, xxvi, 970.
(8) DAGGS, R. J. Journ. Nutrition, 1931, iv, 443.

THE RELATIONS OF THE INTRACRANIAL PRESSURES

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Received for publication May 6, 1933

With the definite abandonment of the idea of equality in pressure between the cerebrospinal fluid and the cerebral veins, following upon the work of Dixon and Halliburton (2) in 1914, current conceptions have acknowledged an independence of the two pressures. In the horizontal position, four-footed mammals (especially the cat and dog) exhibit a pressure of the cerebrospinal fluid which is customarily slightly higher than that in the superior sagittal sinus (7). The fluid's pressure is momentarily affected by rapid changes in the cerebral arterial pressure, but not by slowly effected alterations (1, 2, 7). Changes in the pressure of the cerebral veins are generally held to alter the pressure of the cerebrospinal fluid, always in the same direction and to a lesser extent. Conversely, alterations in the pressure of the cerebrospinal fluid are assumed by many workers (2) to be reflected in the pressure of the cerebral veins, but conflicting reports of this possible effect are found in the literature. The weight of evidence seems to support the view that these two pressures may modify each other.

These generalizations imply that a condition of pressure-equilibrium exists between these two fluids (cerebral venous blood and cerebrospinal fluid) which are separated by a membrane comprised of vascular and meningeal tissues, and that this equilibrium can be shifted by change in either of these pressures. Such alteration of either pressure would, in this interpretation, lead to a new equilibrium characterized by change in the same direction of the other pressure and by a shift in the position of the membrane separating the two fluids.

During an investigation of the pressure-changes occurring in the cerebrospinal fluid on tilting from the horizontal to the two vertical positions (4, 5, 6), many questions of the relationship between cerebral venous pressure and cerebrospinal fluid pressure necessarily arose. It was considered feasible to gain some idea of the character of the pressure-equilibrium between these two intracranial pressures both in the normal horizontal position and during tilting experiments. In a series of etherized dogs in the horizontal position, the cerebral venous pressure was recorded in the superior sagittal sinus and cerebrospinal fluid pressure was measured

by attachment of a 1 mm. open-end manometer to an occipital puncture-needle inserted into the cisterna magna. The subarachnoid pressure was maintained at any desired level by connection of the needle through a three-way stopcock to a reservoir containing Locke's solution. In other experiments the subarachnoid space was entered by a double puncture-needle, to one lumen of which the manometer was attached and to the other, the reservoir. By this procedure the alterations in the subarachnoid pressure, effected by change in height of the reservoir, were communicated to the manometer only through the cisterna magna, thus assuring transmission of the pressure throughout the subarachnoid space.

In this series of horizontal animals, it was quickly found that even profound alterations of the subarachnoid pressure have no effect upon the pressure in the cerebral veins, thus confirming and extending somewhat the observations of Becht (1). Chart 1 gives a graphic record of a typical experiment: changes of the pressure of the subarachnoid space (cerebrospinal fluid pressure) from plus 450 mm. of saline solution to minus 300 mm. resulted in no significant alteration of intracranial arterial pressure nor of the pressure in the sagittal sinus. This lack of effect of change in subarachnoid pressure upon cerebral venous pressure held for all animals in the series during the first two hours of etherization; but after this period of prolonged anesthesia, occasional animals showed slight reactions in intracranial venous pressure from alteration in subarachnoid pressure. The alterations were of small extent—10 to 15 mm. venous pressure-change on subarachnoid pressure-change of 150 mm.—and are interpreted as being due to general fatigue of the animal, particularly of its vasomotor system.

The lack of effect of change in subarachnoid pressure upon cerebral venous pressure (chart 1) does not extend however to the converse of the experimental procedure. Increase in cerebral venous pressure, as effected by pressure upon the veins of the neck of the animal, results in a prompt rise in the pressure of the cerebrospinal fluid, but always to a lesser extent. This phenomenon is well known experimentally (Dixon and Halliburton, 2; Becht, 1) and is employed clinically in the Queckenstedt test, but Becht's observations alone have been given quantitative study. Eleven experiments in our series yielded an average increase of 267 mm. in cerebral venous pressure and of 176 mm. in cerebrospinal fluid pressure, indicating that 65 per cent of the venous pressure-increase is reflected in the pressure of the fluid. If however the data are taken from the levels at which the pressure of the cerebrospinal fluid is raised but slightly over 100 mm. (as is the case on head-down tilting), the average increases become 178 mm. for the sagittal sinus and 112 mm. for the cerebrospinal fluid. Hence within this normal range of pressure-change, 63 per cent of the sagittal pressure-increase is reflected upon the cerebrospinal fluid. This figure is of the same magnitude as that obtained in previous tilting experiments (3) in

which it was shown that 57 per cent of the cerebral venous pressure-change represented the extent of the total vascular reflection (actual and potential) upon the pressure of the cerebrospinal fluid. A similar value was reported

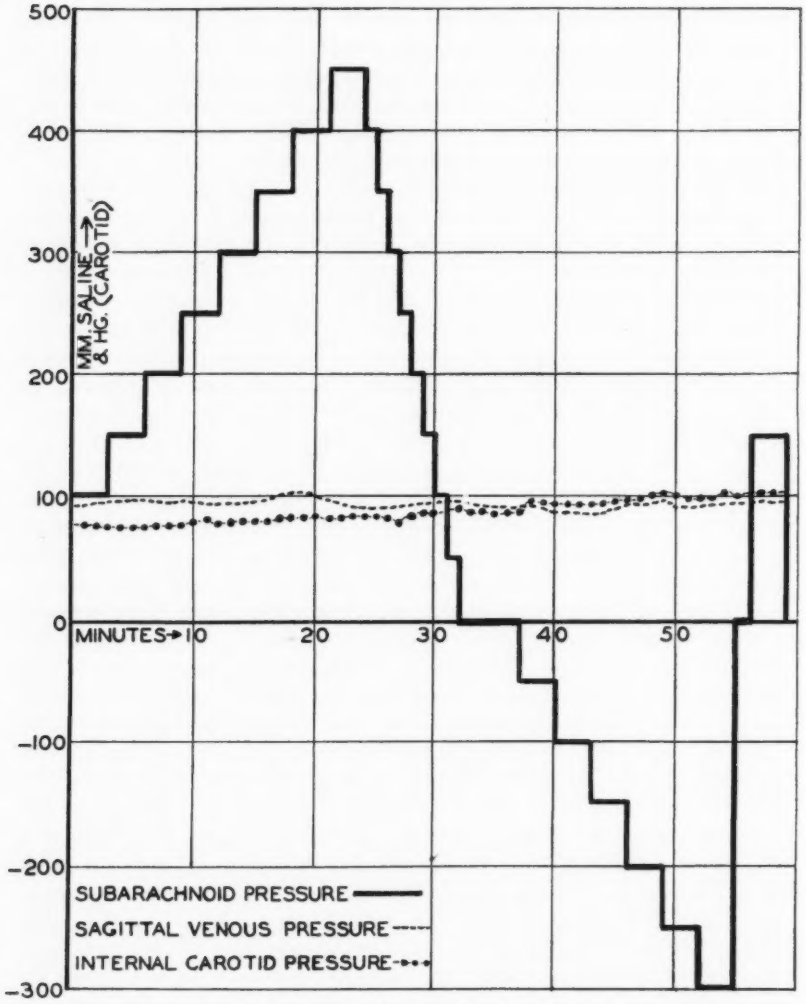

Chart 1. Experiment E 89: Dog with spinal length of 410 mm. under ether anesthesia. Horizontal position. Puncture into the cisterna magna with double needle, one lumen of which was connected to 1 mm. manometer and other to reservoir containing Locke's solution.

by Becht who found that "the rise in the fluid pressure is 61 per cent of the rise in the venous pressure and the fall is 69 per cent of the fall in the venous pressure." The percentage of venous pressure effect in Becht's experiments and in ours is considerably greater than that given by Dixon and Halliburton (one-fourth).

With these data from the horizontal animal in hand, tilting experiments were undertaken on etherized intact animals to ascertain the influence of different pressure-changes in the cerebrospinal fluid upon the pressure-changes in the superior sagittal sinus. The initial procedure consisted in the determination of the average reactions to tilting in the cerebrospinal fluid (using the 1 mm. manometer in which the volume-dislocation is minimal) and in the sagittal sinus, the subsequent attachment of larger manometers with measurement of the average pressure-changes and volume-changes in the cerebrospinal fluid on vertical tilting, and the final re-attachment of the 1 mm. manometer to ascertain the extent of the change in reaction during the period of experimentation. The first half of table 1 gives the results of a typical observation of this kind: it is obvious that different pressure-changes in the cerebrospinal fluid have no effect upon the pressure-changes in the sagittal sinus. These data show in addition a very definite relationship of pressure-change to volume-change in the cerebrospinal fluid. A maximum volume-dislocation of the fluid (2.3 cc.) is apparently achieved in the 8 mm. manometer on head-down tiltings and with the same manometer on tail-down tiltings (1.0 cc.). The employment of larger manometers did not result in the displacement of larger quantities of fluid into or from the animal. The surprising constancy of the pressure-change in the superior sagittal sinus occurred in eighteen out of nineteen dogs; in the one animal a slight but definite decrease in the sagittal pressure-change was encountered with the 8 mm. manometer

A similar series of experiments was performed on dogs immediately post-mortem (with the blood still unclotted). The results of a typical experiment are given in the second half of table 1: here likewise the pressure-changes in the sagittal venous sinus, on tilting, are unaffected by alteration in the pressure-change of the cerebrospinal fluid, as determined in the different manometers. Again a definite relation to pressure-change is shown in the volume of cerebrospinal fluid dislocated into or from the manometers. On the head-down tiltings, the maximum dislocation of fluid (circ. 4.9 cc.) is recorded for the 8 mm. manometer: the larger manometers (10, 12 mm.) gave pressure-changes which were accompanied by volume-changes of the same extent. Similarly in the tail-down tiltings, the maximum volume-dislocation (2.3 cc.) was recorded for the pressure-change in the 6 mm. manometer; the larger manometers all recorded the same volume-dislocation. In these dogs tilted immediately after death, the pressure-changes (both in cerebral veins and in the cerebrospinal fluid) and the

volume-changes in the fluid were considerably greater than in the living intact animals, thus permitting a rough approximation of the extent of vasomotor influence in the living.

This demonstration of the relationship of volume-dislocation to pressure-change in the cerebrospinal fluid in both the living and dead animal, serves to explain the apparent divergence of the diminishing pressure-alterations in the cerebrospinal fluid (when manometers of increasing bore are used) and the constant pressure-change in the sagittal sinus, when considered in relation to the recently advanced thesis (3) of determination of the pres-

TABLE 1

Positional pressure-changes in cerebrospinal fluid and superior sagittal sinus, together with volume-changes of the fluid

CONDITION OF ANIMAL	MANOMETER BORE	HEAD DOWN			TAIL DOWN		
		Pressure-change C.S.F.	Pressure-change sagittal	Volume dislocated C.S.F.	Pressure-change C.S.F.	Pressure-change sagittal	Volume dislocated C.S.F.
	mm.	*mm.*	*mm.*	*cc.*	*mm.*	*mm.*	*cc.*
Living dog, E99	1	185	237	0.321	126	204	0.219
	4	102	230	1.163	64	204	0.736
	6	62	229	1.779	33	204	0.947
	8+	33.5	234	2.258	15.5	201	1.045
	10−	30.5	233	2.173	14.5	200	1.033
	12	20.5	235	2.337	9.0	199	1.026
	1*	185	235	0.321	110	198	0.191
Dog, immediately after death, E68	1	243	325	0.422	234	299	0.406
	4	208	328	2.392	141	304	1.622
	6	145	327	4.162	79	298	2.267
	8+	73	329	4.920	32	295	2.167
	10−	68	325	4.845	33	296	2.350
	12	43	328	4.902	20	297	2.280

* 1 mm. manometer replaced after other manometers were used in series.

sure-change in the cerebrospinal fluid by one of the two factors (meningeal element and vascular reflection). For on the basis of this hypothesis, it might be thought that the similar pressure-changes in the sagittal sinus should be accompanied by the same pressure-changes in the cerebrospinal fluid, whatever the bore of the manometer. The thesis obviously can hold only when the volume-dislocation is minute enough to be easily accommodated by the animal; the total quantity of cerebrospinal fluid is so small that a limit to volume-displacement is soon reached even though the intradural blood-volume varies reciprocally. The head-down extrusion of fluid is ofttimes larger than the tail-down introduction of fluid from the manometer—a phenomenon apparently connected with the relative pressure-

changes in cerebrospinal fluid and in cerebral venous system on the two types of vertical tilting. While the volume of cerebrospinal fluid in these animals is relatively small, the blood-volume in its effective hydrostatic column is large: the apparent volume-compensation in the cerebral veins is not great enough to affect measurably the height of the effective venous column. In consequence of this great difference in volumes of blood and cerebrospinal fluid, the pressure-alterations in the sagittal sinus on tilting remain constant even when on the other side of the membrane the pressure-changes in the cerebrospinal fluid are of decreasing magnitude.

These data, indicating a lack of influence of subarachnoid pressure-change upon sagittal venous pressures and a potent influence of cerebral venous pressure-change upon the pressure of the cerebrospinal fluid, permit an interpretation of the kind of pressure-equilibrium which exists between these two fluids. Any intracranial venous pressure-change results in change of the cerebrospinal fluid pressure, about $\frac{1}{3}$% of the venous pressure-change being effective in the fluid when the fluid-dislocation is minimal. With the experimental increase of cerebrospinal pressure brought about by the introduction of fluid into the subarachnoid space, a certain diminution of intradural venous volume must follow; but the pressure in these cerebral veins remains unaltered. Conversely when the pressure of cerebrospinal fluid is reduced mechanically or by tilting, the cerebral venous bed apparently compensates by dilatation, the venous walls expanding and permitting an increase of intradural venous volume, though maintaining in these expanded channels the same pressures. Under both of these conditions, the venous walls come to rest (i.e., to equilibrium) at a point determined by the new subarachnoid pressure. There is however a distinct limitation to this process—the limitation of volume-dislocation which is shown experimentally both in the living and dead animal. When this limit of volume-readjustment by the venous bed is reached, the venous walls must remain in a "steady state," irrespective of further alteration in the pressure on their outer sides.

The type of pressure-equilibrium between cerebral veins and cerebrospinal fluid therefore seems to be that of an elastic membrane separating two fluids which are normally under almost identical pressures but which can exist under very different pressures. The two fluids are, however, of very unequal volume—that of the blood being far greater than that of the fluid. The venous blood is not necessarily confined within the cranium and is under direct or indirect vasomotor control; the cerebrospinal fluid is enclosed within fairly rigid membranes and cannot leave its cranio-vertebral container with any great rapidity. The vasomotor system seems capable of maintaining identical intracranial venous pressures in the horizontal position by reciprocal volume-changes even when subarachnoid pressures are markedly altered; and it seems capable also of maintaining

the same positional pressure-changes even when the positional pressure-changes and volume-changes in the cerebrospinal fluid are of far different magnitude. On the other hand, any venous pressure-change seems to be reflected upon the cerebrospinal fluid pressure through the mechanism of cerebral venous (and capillary) volume-change, but a limit to the totality of this venous volume-compensation is indicated. Apparently in a dog of 400 to 450 mm. spinal length (occiput to last lumbar spine), a volume-dislocation in the cerebrospinal fluid approximating 0.3 cc. can usually be accommodated without affecting the positional pressure-change in the cerebrospinal fluid.

SUMMARY

In the horizontal etherized dog, abrupt alterations in the pressure within the subarachnoid space were found to be without effect upon the sagittal venous pressure, but pressure-changes within the cerebral veins caused pressure-changes within the cerebrospinal fluid. On tilting from the horizontal to the two vertical positions, the pressure-alterations in the sagittal sinus remained constant even when the pressure-changes in the cerebrospinal fluid were of different magnitudes. A relationship between the volume of cerebrospinal fluid dislocated and the pressure-changes was shown.

An interpretation of the type of pressure-equilibrium between cerebral veins and cerebrospinal fluid is given.

REFERENCES

(1) Becht, F. C. This Journal, 1920, li, 1.
(2) Dixon, W. E. and W. D. Halliburton. Journ. Physiol., 1914, xlviii, 128.
(3) Flexner, L. B. and L. H. Weed. This Journal, 1933, civ, 681.
(4) Weed, L. H. Arch. Surg., 1929, xviii, 1049.
(5) Weed, L. H. and L. B. Flexner. Bull. Johns Hopkins Hosp., 1932, l, 196.
(6) Weed, L. H., L. B. Flexner and J. H. Clark. This Journal, 1932, c, 246.
(7) Weed, L. H. and W. Hughson. This Journal, 1921, lviii, 101.

TEMPERATURE STUDIES IN NORMAL AND SUPRARENALECTOMIZED RATS

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Received for publication April 10, 1933

The relationship between the hormones of the suprarenal gland and the regulation of body temperature in the mammalian body has been a debatable question for many years. Cannon and Querido (5) emphasized the importance of the medullary secretion of the suprarenal gland in the chemical control of body temperature and the appearance of the shivering mechanism when that hormone was lacking. In 1929, Wyman and Tum Suden (21b) reported a fall in rectal temperature in suprarenalectomized rats and a delayed recovery following an exposure to a cold room for two hours. A definite correlation was shown between these phenomena and the degree of cortical insufficiency. These authors thus concluded that both the suprarenal cortex and the medulla were related to the normal temperature balance of the body. This relation, it was pointed out, was an indirect one being secondary to metabolic disturbances produced by the suprarenal insufficiency. Hartman (12b) and his colleagues concluded from their work that it is not the medullary secretion but the hormone of the suprarenal cortex that is essential for temperature regulation. The following investigation was undertaken in an attempt to study the relative importance of the suprarenal cortex in maintaining body temperature. The experiments were divided into 1, a statistical survey of the biological range of colonic temperature in rats with reference to age, sex, weight, strain, season and time of day, and 2, a study of this temperature after complete suprarenalectomy and after short periods of etherizations. A preliminary report of this research has been published (14b).

PROCEDURE AND RESULTS. 1. *Biological range of colonic temperature in rats.* Albino rats ranging in age from 2 to 20 months were picked at random from three different strains. Single determinations of the colonic temperature and body weight were made on 193 males and 438 female rats during a period of 18 months. This interval was long enough to observe possible seasonal variations. In a small group of females, vaginal temperatures and smears were also recorded in an attempt to find a correlation to the different phases of the oestrual cycle.

All temperature determinations were made with the use of a thermo-

couple galvanometer arrangement sensitive to 0.05°C. The warm junction was strengthened by fastening it with insulating cement to a copper wire; the ends were rounded with a drop of solder and insulated. The rigidity of this support was sufficient to pass the wire by a lump of feces but not enough to pierce the wall of the colon.

While the rat was held comfortably by an assistant, the couple was inserted into the colon 5 to 6 cm. from the anal opening and into the vagina 1 to 2 cm. from the surface of the body. The temperature reading was easily taken in less than 30 seconds. Struggling was rarely encountered during this short interval and fecal masses presented no difficulty.

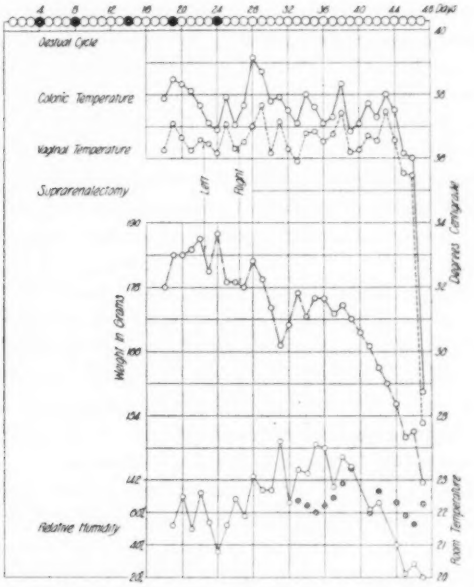
The distribution of the normal colonic temperature of female rats obtained from three different strains is found to be as follows. The most probable temperature is 38.2°C. with one-half of all determinations lying in the range of 37.8°C. to 38.7°C. The extreme limits of all the temperatures are 36.5°C. and 39.5°C. Slight variations were noted from day to day with no apparent relation to the hour. The vaginal temperature was about 1°C. below the colonic temperature and closely followed the daily fluctuations of the latter. This lower temperature is undoubtedly due to the proximity of the vaginal cavity to the surface of the animal's body and hence approaches the skin temperature. Vaginal temperatures taken during the various phases of the oestrual cycle showed no correlation between temperature and the stage of the cycle (graph 1). The range of colonic temperature in male rats is practically the same as in the females with the most probable temperature slightly lower. In both males and females, the temperature distribution is the same for variations in weight, age, season and time of day.

2. *Colonic temperature of rats following complete suprarenalectomy.* Smears of the vaginal contents of all females, and colonic temperatures and body weights of 75 male and female rats were made for a period of 9 to 14 successive days prior to any treatment. These observations established a normal curve of the temperature, weight and oestrual history of the animals. Of these, 53 were completely suprarenalectomized by a two stage lumbo-dorsal approach allowing at least 3 days between operations; the other 22 were unilaterally suprarenalectomized and kept as controls. An additional series of controls received the following treatment during an interval of three weeks; splenectomy and a unilateral removal of the thyroid, suprarenal, ovary and uterus. Both the ordinary antiseptic and rigid surgical aseptic techniques were employed. Daily readings were continued until the death of all experimental rats.

The survival period of 164 rats taken from three different strains was treated statistically. The most probable survival period is 15 days with one-half of the number of animals dying between the 12th and 18th days. The extreme limits of survival are 7 and 29 days. Ninety-five per cent

of the rats died after complete suprarenalectomy. In agreement with previous and more recent investigations (9, 10, 14a), the rat is thus no exception to the fatal effects of double adrenalectomy.

The completely suprarenalectomized rats after the last operation soon showed definite objective signs of cortical insufficiency particularly an asthenia, loss in body weight and oestrual disturbance in females. The colonic temperature, however, remained within the normal range until the impending death of the animal; in about one-third of the cases, it declined



Graph 1. The effect of complete suprarenalectomy on the daily records of the body weight, oestrual cycle and colonic and vaginal temperatures of an albino rat. Note particularly the normal temperature readings until 24 hours before death of the animal from suprarenal insufficiency.

rapidly in the terminal stages 24 to 72 hours before expiration. Control animals showed a normal colonic temperature throughout the period of observation. It is thus concluded that rats can maintain their body temperature for a relatively long period in the complete absence of their suprarenal glands. It is only in the terminal stages in some rats that the body temperature declines.

These results are illustrated in graph 1 by typical observations on a suprarenalectomized female rat. After complete suprarenalectomy, the

oestrual cycle was completely inhibited as reported in a previous paper (14a). The colonic and vaginal temperatures showed slight fluctuations from day to day but remained constant within the normal range until 24 hours before expiration. During this terminal period, the temperature began to decline and continued to do so until the rat died. The body weight decreased consistently after cortical insufficiency became manifest. The variations in room temperature and relative humidity had no influence upon the other events.

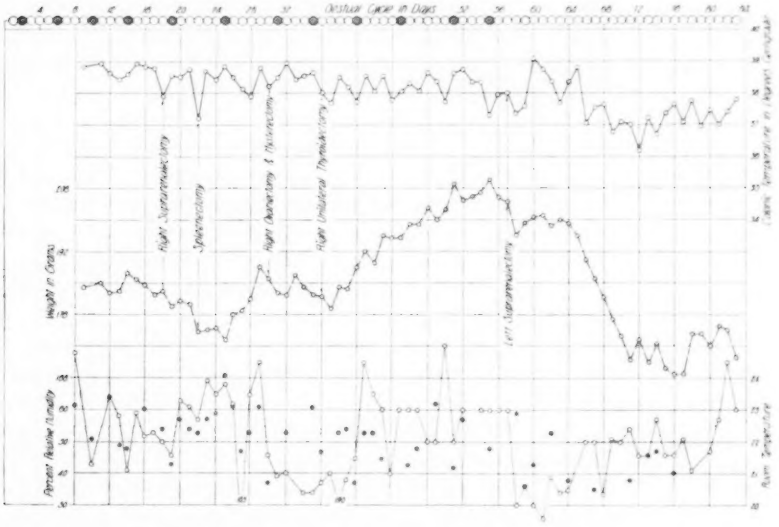
The pre-mortal fall in colonic temperature in suprarenalectomized rats was precipitous in some cases and so consistently accompanied the terminal stages of suprarenal insufficiency that it was regarded as an unmistakable sign of approaching death. The usual decline was noted within 24 hours before expiration. The subnormal temperatures taken from animals in a moribund or semicomatose condition ranged from 35.4° to 24.1°C. but no correlation was found between the moribund condition of the animal and any given subnormal temperature. Some became comatose at 35.7°C., others at 25.0°C. while a few were apparently normal at 34.0°C. The temperature of the animals seemed to be the result of approaching death and not the cause of it.

Graph 2 illustrates the constancy of the colonic temperature and oestrual cycle of a female rat following a series of control operations. The body weight shows a normal increase during the period of observation. After the removal of the second suprarenal gland, changes in the body weight, oestrual cycle and colonic temperature in this animal were similar to those in other bilaterally suprarenalectomized rats. The effect of surgical trauma is thus a negligible factor in these experiments.

3. *Colonic temperature of suprarenalectomized rats following a short etherization.* The above results show that rats maintain their normal body temperature for a long period after complete suprarenalectomy. The relative ability of these animals to withstand an experimental reduction of body temperature long before the onset of terminal symptoms was next studied. The method adopted consisted of inducing surgical anesthesia by the cone method for definite intervals. The animals were tied down upon an operating board and the thermo-couple inserted into the colon. Readings at 2.5 minute intervals for a period of 10 to 20 minutes were recorded to establish a control level of temperature. Occasionally the animals struggled and produced slight increases in temperature readings. These, however, were less than the changes from day to day and so were considered negligible. At the end of the control period, ether was administered. Temperature readings were recorded throughout the etherization and until the recovery or impending death of the animal. Etherization for 5, 8, 10, 12, 15, 20 and 25 minutes was studied on 34 rats; for any of these periods, the behavior of individual rats varied so that no strict correlation

to the time of ether administration could be noted. However, a 20-minute etherization had a greater and longer effect than a 10-minute one. The 10 minute etherization was arbitrarily adopted for the test since it corresponded to the time of anesthesia during a suprarenalectomy.

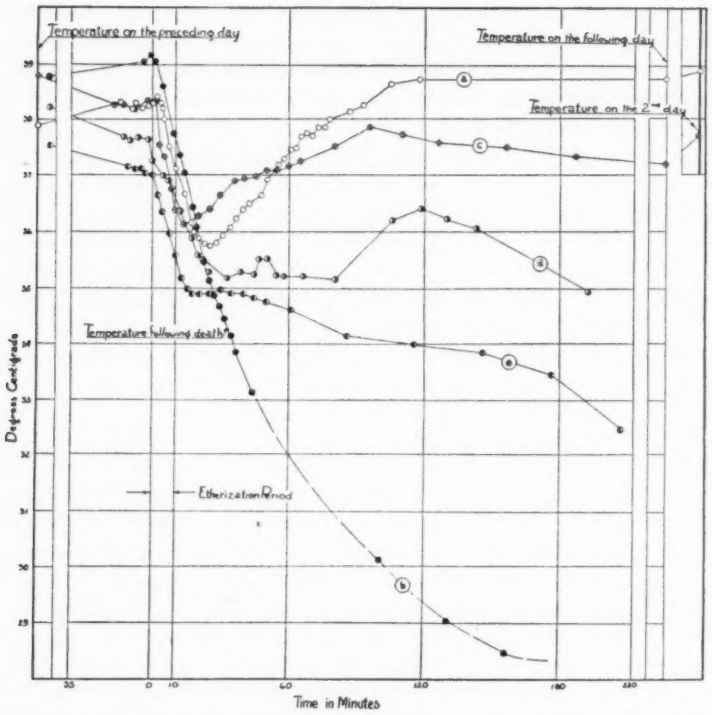
Graph 3a shows the average course of the colonic temperature in 23 unoperated normal control rats preceding and following a 10-minute etherization. During a 15 minute interval preceding the etherization, the colonic temperature fluctuated within a 0.2°C. range. An immediate and abrupt fall in temperature began with the administration of the ether



Graph 2. The relation between the surgical trauma following a series of operations and the daily readings of body weight, oestrual cycle and colonic temperature of an albino rat. Note that ill effects are seen only after the removal of the second suprarenal. Room temperature and relative humidity within the range noted are without effect.

in four-fifths of the cases; in about one-fifth of the etherizations, an increase of 0.1° to 0.5° not exceeding three minutes in duration preceded the fall. The rate of temperature decline during the etherization was quite constant for all rats and runs. At the end of a 10-minute etherization period, the temperature fall was 1.5° to 2.5°C; the maximal depression of the temperature ranged from 2.0° to 4.0°C. and was attained 20 to 30 minutes after the beginning of etherization. After the maximal fall, the temperature rose gradually to or slightly above the preliminary control level in 90 to 120 minutes. The temperature rise showed many individual variations

but in all cases, the normal level was attained within a 2 hour period. Graph 3b also shows the course of colonic temperature in a rat which was killed during the first few seconds of etherization. The decline in temperature in the dead rat was approximately the same as during the adminis-



Graph 3. Changes in the colonic temperature of normal control and completely suprarenalectomized rats following a ten minute etherization. The effects noted are: *a*, normal temperature recovery; *c*, delayed temperature recovery; *d*, attempted recovery with subsequent death, and *e*, no attempt at recovery followed by death. Curve *b* shows the fall in colonic temperature of a rat that was killed during the first few seconds of etherization.

tration of ether in a living rat and continued at a regular rate until room temperature was reached.

The administration of ether for 20 minutes showed approximately the same rate of temperature fall during the etherization as for the 10 minute interval, a total depth of 3.5°C. occurring in 35 minutes. After the minimal temperature was reached, normal temperature returned within

150 minutes. A longer period of etherization tended to prolong the phases and to increase the effects observed in the 10 minute etherization curve.

The temperature curves of single ether determinations of 27 unilaterally suprarenalectomized rats were essentially similar to those of etherized unoperated controls. A slight difference, however, was noted in the tendency towards a more gradual recovery although this was completed in all cases within 2 hours. Repeated etherizations for 2 to 4 successive days of these rats and for 2 to 6 successive days of unoperated rats presented no significant change in temperature behavior.

A group of 3 unoperated control female rats was etherized for 10 minutes on five successive days. On the 6th day, the left suprarenal gland was removed from these animals. The 10 minute etherizations were continued on the following 4 days. Temperature readings were recorded during each etherization for the 10 successive days. All animals showed a normal recovery of colonic temperature each day. There were no oestrual disturbances throughout the period of 10 days, and only a slight and temporary loss in body weight was noted. The removal of the remaining gland resulted in typical symptoms of suprarenal insufficiency and death of these rats. The ability of the rat to recover from an artificial decrease in body temperature of 2.0° to 4.0°C. is not impaired by successive daily etherizations so long as one suprarenal gland remains intact.

Bilaterally suprarenalectomized rats were similarly etherized for 10 minutes on different days after the second gland was removed; only those rats which showed a normal and constant pre-etherization temperature level were employed. In all instances, at least one etherization had been given before complete suprarenalectomy to serve as a control record for that animal. The results of this procedure applied to 37 suprarenalectomized rats may be conveniently grouped as follows:

1. *Rats that showed a normal recovery.* After the etherization, the course of the colonic temperature in 8 rats was essentially the same as that shown by normal control rats (graph 3a). Normal temperature in all of these rats returned to the control level within $1\frac{1}{2}$ to 2 hours.

2. *Rats that showed a delayed recovery.* This group consisted of 4 rats that showed an incipient fall in colonic temperature upon etherization that was indistinguishable from that seen in normal control rats. However, all rats required from 3 to 48 hours (graph 3c) to regain their normal body temperature.

Animals in groups 1 and 2 showed little or no impairment in their ability to recover from an experimental fall in body temperature of 2° to 4°C. The etherization of these rats was applied in the range of 2 to 28 days after complete suprarenalectomy. All rats eventually died of suprarenal insufficiency although they had shown a recovery of colonic temperature to normal level. Death occurred on the 4th to 14th day after etherization.

3. *Rats that attempted a recovery but died.* After beginning the etherization, the succession of the colonic temperature readings in 17 suprarenalectomized rats during the first 20 to 40 minutes followed the decline observed in control rats for this same interval. After the minimal temperature had been reached, the return to a normal level was sluggish and incomplete; it varied in extent from 0.3° to 2.0°C. This slight elevation above the minimal lingered from a few minutes to several hours and was followed by a gradual and persistent decline (graph 3d). After the progressive fall in temperature was established, the rat was on its way to death. All rats in this group died after the etherization. The short period of etherization was applied 1 to 9 days after removing the second suprarenal gland and the number of etherizations per rat in that period ranged from 1 to 5. Twelve rats died on the day of etherization; the remaining five were found dead on the following morning.

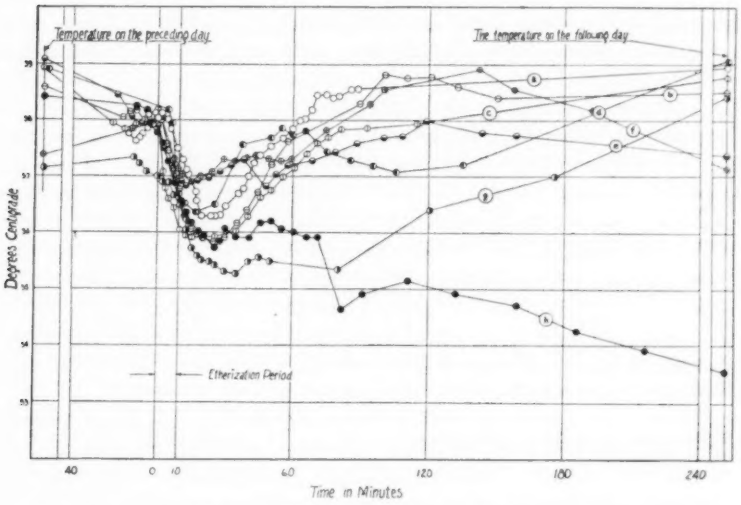
4. *Rats that made no attempt to recover.* Graph 3e illustrates the progress of the colonic temperature of 8 etherized rats 3 to 29 days after complete suprarenalectomy showing the usual incipient fall and its continuance after the administration of ether. However, in contrast to normal animals and to the experimental rats of the first three groups, the temperature continued to fall until a moribund state and death of the animal occurred. No attempt at temperature recovery was noted and all animals died within 1.5 to 48 hours after etherization.

All animals of groups 3 and 4 showed a subnormal temperature which never returned to the control level after etherization was begun. The failing condition of these rats showed no relation to any given temperature reading. These animals exhibited a complete and fatal impairment in their ability to survive a fall in body temperature.

Graph 4 shows the behavior of the colonic temperature of an individual rat under etherizations during different phases of suprarenalectomy. The fall in temperature from etherization was smaller than usual and ranged from 1.1° to 2.3°C.; however, this was characteristic for the animal and showed up under subsequent etherizations. Curve 4a illustrates the course of the colonic temperature of a rat subjected to a 10 minute etherization on the day it was brought to the laboratory. Curves 4b and 4c record the progress of temperature during etherization 1 and 4 days after the first suprarenal gland was removed. Curve 4d is the temperature change during the removal of the second suprarenal gland; 11 days had elapsed between the suprarenalectomies. Curves 4e, 4f, 4g and 4h show the succession of temperature during the etherization on the 2, 4, 6 and 8th day after complete suprarenalectomy. In the 2nd and 4th days, the recovery is at a normal rate and cannot be distinguished from the pre-operative runs. On the 6th day, the rate of return is much slower than previously but still complete. On the 8th day, the maximal depression of 2.3°C. is followed

by a slight (0.4°C.) elevation for about 60 minutes which gives way to a progressive decline ending in death. Daily temperature readings were constant within the normal range throughout the entire period of observation.

The results of short etherizations performed on unoperated, unilaterally and completely suprarenalectomized rats are summarized in table 1. A total of 115 ether determinations were made on 50 rats with one or both suprarenals present. In most cases, the colonic temperature readings were recorded before, during and after the removal of one gland. All



Graph 4. Changes in the colonic temperature of an albino rat following a ten minute etherization before and on different days after complete suprarenalectomy. See text for details.

these animals showed a typical normal recovery from the 10 minute etherization.

A group of 37 completely suprarenalectomized rats was used on which a total of 81 ether determinations was made. Several "ether runs" on successive and alternate days after complete suprarenalectomy were recorded on individual rats; in other cases only one determination was made in the post-suprarenal period. Temperature readings during the removal of the 2nd suprarenal were also taken in a few cases. The effect upon the colonic temperature of ether during suprarenal removal and an ordinary "ether run" was of equal magnitude.

A survey of the figures in table 1 reveals the wide, individual variation of the behavior of the colonic temperature of rats to a 10 minute etherization given on different days after a complete suprarenalectomy. The failure of the temperature to return to normal can occur on practically every day that ether is given after suprarenal extirpation. This may be accounted for by an abortive attempt of accessory cortical tissue to secrete an adequate amount of hormone, or possibly by the assumption of individual differences in storage supply of the hormone. The data show that there is at least a slight tendency toward a correlation between the day after removal of the 2nd suprarenal and the inability of the animal to recover from an experimental reduction of body temperature (2–4°C.). The experience with our rats suggests that in most animals this occurs at about the 8th day. Enough experiments have not been performed to determine

TABLE 1

Effects following a ten minute etherization on the colonic temperature of unoperated, uni-laterally and completely suprarenalectomized albino rats

EFFECTS ON COLONIC TEMPERATURE FOLLOWING 10 MINUTE ETHERIZATION	NUMBER OF ETHERIZATIONS								
	Unoperated rats	Unilateral suprarenalectomized rats	Days after complete suprarenalectomy						
			0-2	3-4	5-6	7-8	9-10	11-12	14-19
1. Normal recovery, 1–2 hours	50	65	26	5	3	1	1		2*
2. Delayed recovery, 3–48 hours			1	5	2	1	1		
3. Attempted recovery followed by death			4	4	4	2	1		2
4. No attempt at recovery followed by death			2	2	2	6	1	1	2

* Probably due to functional suprarenal accessories or remnants.

the frequency distribution in regard to the day after which no suprarenalectomized rat in pre-terminal stages will be able to survive an induced fall in body temperature.

It was next considered advisable to determine the efficacy of suprarenal extracts in restoring the ability of an adrenalectomized rat to survive an "ether temperature fall." A small amount of standardized Swingle-Pfiffner extract was kindly furnished by Parke, Davis & Co. Two completely suprarenalectomized rats, after definite symptoms of glandular insufficiency appeared, were injected subcutaneously twice daily with extracts for 19 to 20 days. The symptoms disapperaed soon and the rats, in an apparently normal condition, were given a 10 minute etherization on the last day of injection, or 27 to 28 days after complete adrenalectomy. The injected rats at this time had been kept alive for almost twice the

average survival period noted for rats of the strains used. The colonic temperature in both rats showed a normal return following etherization. The injections were then stopped and the rats died 4 and 5 days later.

DISCUSSION. In a study of this nature, it seemed of utmost importance first to have available a quick, accurate and convenient method for the determination of body temperature. The thermocouple arrangement used in this work fulfilled all these requirements and was very suitable for obtaining colonic temperature in rats. In contrast to the use of the chemical (12b) or the certified clinical (21b, c) thermometers, the success of the thermocouple method was not impeded by any difficulty of inserting the instrument or by the presence of fecal masses in the colon. Struggling was a very insignificant factor during the rapid period of temperature determination.

Secondly, the necessity for establishing a biological range of temperature on a statistical basis seemed apparent from the wide normal variations noted in the early part of this investigation as well as in the reports of Lee, Vincent, Hartman, Donaldson, Wyman and others. It is unequivocally admitted that body temperature is one of the most constant biological features of the warm-blooded animals. In the white rat, however, temperature determinations show daily fluctuations with no apparent correlation to weight, age, season and time of day. This may be explained in part by the fact that the rat is only imperfectly homoiothermic and tends to follow roughly the environmental temperature (7, 11, 19). The variations noted, however, did not follow the small changes in temperature of the animal room which was regulated by a thermostat. This is in agreement with the work of Schear (16) who found that body temperature remains unaffected by changes in environmental temperature ranging from $-8°$ to $+34°$C. The higher temperature noted in female rats indicates possibly that there is a greater thermolysis in the males since it was found by Benedict and Macleod (3) that the latter have a higher basal metabolism than the females. The biological range of colonic temperature has served to define the limits of normal variation in rats. This is of particular importance when studying deviations from the normal following experimental procedures.

The course of symptoms in completely suprarenalectomized laboratory animals is characterized by being relatively short. This fact alone seems to make it imperative to make daily observations throughout the entire experiment in order to gain a true insight of the progress of symptoms. We have kept this foremost in mind particularly in studying colonic temperature.

The appearance of certain definite symptoms that follow cortical insufficiency such as anorexia, cachexia and progressive asthenia is now universally recognized. However, there is some disagreement concerning

changes in body temperature. Thus, a fall to a subnormal level is seen early after complete adrenalectomy in the cat (8, 15) and rat (1, 6, 21a) and only in the terminal stages of glandular insufficiency in the rat (9, 10), cat (18) and dog (2, 17). Our findings, similar to those reported by Gaunt, and Firor and Grollman, clearly show that the body temperature of completely suprarenalectomized rats remains always within the normal range until the terminal stages intervene.

The undisturbed body temperature may be explained by assuming that the hormone of the suprarenal cortex bears an important relationship to the maintenance of body temperature (12b) and that there is possibly a progressive decrease in the supposed storage of the hormone until the minimal threshold is reached in the final stages of life. Or, it may be assumed that the fall in temperature during the terminal stages is due to the cumulative effects of regressive changes in the principal tissues of the body. That the former view is untenable at the present time at least is supported by the failure of some to demonstrate the active principle in question from tissues other than the suprarenal gland (4). The latter explanation, however, seems to be more appropriate and in greater physiological harmony with the researches already reported on blood chemistry changes, central nervous system disturbances, etc., of suprarenalectomized animals. In short, the extreme intoxication in the terminal stages of insufficiency may be quite logically attributed and traced to the various tissues of the body. This view is based essentially upon the contention that the cortical hormone is a general tissue hormone (12c, 20). We believe, therefore, that the cortical secretion of the suprarenals is related to body temperature only in so far as its presence maintains the normal physiological state of the body tissues. It is not a temperature regulating hormone any more than the thyroid or pancreatic secretions are. Its absence or sub-threshhold secretion, in our opinion, impairs first the function of tissues showing the highest rate of activity and then others in order. This modification of the "general tissue hormone" theory would serve to explain the complexity of symptoms and their appearance along with the inevitable tissue intoxication of the terminal stages.

The use of ether in producing an experimental fall in body temperature seemed to us of especial value in this work. Aside from its ease of administration, it proved to be an ideal agent for taxing the temperature regulating mechanism of the animal's body. That the ether depressed this mechanism in a suprarenalectomized rat to a temperature level approaching that of a dead animal may be concluded from the fact that the rate of temperature fall in both cases is approximately the same. The pharmacological action of ether involved directly or indirectly the metabolism of the principal tissues of the body and thus became intimately related to the suprarenal cortical hormone. Briefly, the method of etherizing albino rats for a

short period enabled us to study the degree of tissue impairment and the concomitant inability of the suprarenalectomized animal to recover from an induced fall in body temperature. Such a procedure, it is suggested, may be employed to standardize extracts of the suprarenal cortex substituting immature for the adult suprarenalectomized rats.

SUMMARY AND CONCLUSIONS

1. A thermo-couple galvanometer arrangement has been found suitable and accurate for the determination of colonic and vaginal temperature in albino rats.

2. A statistical study of the colonic temperature of adult female rats obtained from 3 different colonies shows a most probable temperature of 38.2°C. with one-half of all determinations lying in the range 37.8° to 38.7°C.; the most probable temperature in male rats is slightly lower. The vaginal temperature closely follows the fluctuations in colonic temperature and is always 1°C. below it. The different stages of oestrum show no correlation to the vaginal temperature. In both males and females, the temperature distribution is the same for variations in weight, age, colony, season and time of day.

3. The average survival period noted in 164 suprarenalectomies is 15 days; 95 per cent of the rats obtained from three different strains died from suprarenal insufficiency.

4. Following complete suprarenalectomy, the colonic temperature in rats remains normal for a relatively long period until 24 to 72 hours before death; in some cases, the fall in temperature in the terminal stages was precipitous in nature. No relationship was found between the semicomatose condition of a dying animal and any given temperature reading. All control animals showed a normal temperature throughout the experiment. Surgical trauma was a negligible factor in our work.

5. A ten minute etherization has been used as an experimental method for inducing a fall in body temperature. All unoperated and unilaterally adrenalectomized rats showed a normal return of colonic temperature within 2 hours. Successive daily etherizations of these animals did not influence their ability to recover from an experimental decrease in body temperature.

6. The behavior of the colonic temperature of suprarenalectomized rats after a ten minute etherization may be grouped as follows: 1, normal recovery; 2, delayed recovery; 3, attempted recovery with subsequent death, and 4, death with no attempt made at recovery. The ability to recover from an experimental fall in body temperature in rats of groups 3 and 4 was definitely impaired. A few results suggested that the longer the period after complete suprarenalectomy, the weaker the resistance of the animal toward an induced fall in body temperature.

7. The cortical secretion of the suprarenals is related to the maintenance of body temperature only in so far as its presence is concerned with the normal physiological state of body tissues.

REFERENCES

(1) Adler, A. A., M. Tausk et al. 1932. Acta Brev. Neerl., ii, 150.
(2) Banting, F. G. and S. Gairns. 1926. This Journal, lxxvii, 100.
(3) Benedict, F. G. and G. Macleod. 1929. Journ. Nutr., i, 367.
(4) Britton, S. W. and H. Silvette. 1931. This Journal, xcix, 15.
(5) Cannon, W. B. and A. Querido. 1924. Proc. Nat. Acad. Sciences, x, 245.
(6) Carr, J. L. 1931. Proc. Soc. Exper. Biol. and Med., xxix, 128.
(7) Donaldson, H. H. 1924. Mem. of Wistar Inst. of Anat. and Biol., no. 6, 2nd ed., 151.
(8) Elliott, T. R. 1914. Journ. Physiol., xlix, 38.
(9) Firor, W. M. and A. Grollman. 1933. This Journal, ciii, 686.
(10) Gaunt, R. 1933. This Journal, ciii, 494.
(11) Goto, K. 1923. Biochem. Zeitschr., cxxiii, 107.
(12a) Hartman, F. A. 1931. Proc. Soc. Exper. Biol. and Med., xxviii, 702.
(12b) Hartman, F. A., K. A. Brownell and A. A. Crosby. 1931. This Journal, xcviii, 674.
(12c) Hartman, F. A., K. A. Brownell and J. E. Lockwood. 1932. Endocrinol., xvi, 521.
(12d) Hartman, F. A., J. E. Lockwood and L. M. Lockie. 1932. Proc. Soc. Exp. Biol. and Med., xxix, 409.
(13) Lee, M. O. 1926. This Journal, lxxviii, 246.
(14a) Martin, S. J. 1932. This Journal, c, 180.
(14b) Martin S. J. and Frank Maresh. 1932. This Journal, ci, 74.
(15) Pfiffner, J. J. and W. W. Swingle. 1931. Endocrinol., xv, 335.
(16) Schear, E. W. E. 1932. This Journal, xcix, 555.
(17) Stewart, G. N. and J. M. Rogoff. 1925. Proc. Soc. Exper. Biol. and Med., xxii, 394.
(18) Swingle, W. W. and J. J. Pfiffner. 1930. Science, lxxii, no. 1855, 75.
(19) Vincent, S. 1925. Quart. Journ. Exper. Physiology, xv, 319.
(20) Webster, B., J. J. Pfiffner and W. W. Swingle. 1932. This Journal, xcix, 710.
(21a) Wyman, L. C. 1928. This Journal, lxxxvi, 528.
(21b) Wyman, L. C. and C. Tum Suden. 1929. This Journal, lxxxix, 362.
(21c) Wyman, L. C. and C. Tum Suden. 1932. Proc. Soc. Exper. Biol. and Med., xxx, 61.

THE TRANSPORT OF SPERMATOZOA IN THE DOG

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Received for publication April 3, 1933

Recent investigations tend to show that the normal duration of life of mammalian spermatozoa in the female tract is a matter of hours, not days. In the migration of spermatozoa up the uterus to fertilize a single or several eggs, it is inevitable that, of the millions which start this journey, all but a few must perish. It is essential that this journey be relatively swift, otherwise all the spermatozoa will have been destroyed before fertilization can take place.

With innovations in the practices of breeding domestic animals, i.e., the introduction of artificial-insemination technique, etc., it is imperative that we learn more concerning how long spermatozoa live outside and in the body, how they are transported in the uterus, how their metabolic rate is altered by changes in temperature, in pH of the medium, and so on. Prior to studies of a similar nature on the domestic ox, the transport of spermatozoa was studied in the dog, by reason of availability and the low cost of experimental material.

In Bischoff's (1845) classic work, *Die Entwicklungsgeschichte des Hunde-Eis*, mention is made of certain experiments pertinent to the present discussion. This careful investigator killed female dogs at definite intervals after coitus and examined the uterus, oviducts and ovarian bursa for presence of spermatozoa. In one dog (loc. cit., p. 14) immediately after coitus, he found spermatozoa in the entire uterus as far as the apex of the horns, but none in either oviduct. In another dog, forty-five hours after the first coitus, both horns of the uterus were found to be filled to the apex with spermatozoa, but the Fallopian tubes contained no trace of spermatozoa whatever. Several dogs ($18\frac{1}{2}$, $19\frac{1}{2}$, 20 hours after the first coitus) showed spermatozoa in the entire uterus and even in the Fallopian tubes. On the basis of these and many other observations, Bischoff drew the following conclusions (p. 16):

1. The semen, in the case of the very first covering, gets up through the cervix into the utmost parts of the cornu of the uterus.

2. The semen, in the course of the following hours, gets into the oviducts, and can get as far as on the surface of the ovary.

Amantea (1924) reports sperm recovered from a uterine fistula eleven minutes after copulation; Whitney (1927) demonstrated the presence of sperm in the ovarian bursa of one bitch twenty minutes after the beginning of copulation.

Until Hartman and Ball's paper (1930) appeared, little thought had been given to the possibility that spermatozoa arrive at the ostium of the oviduct by means other than their own motion. These workers demonstrated that in one species, the rat, spermatozoa arrive en masse at the apex of the uterus horns in less than two minutes. They concluded that "The transport of sperms, at least in the uterus of the rat, is a matter not of hours or of minutes, but of seconds."

By means of the uterine fistula technique, Florey and Walton (1932) have confirmed the work of Hartman and Ball on the rat. Furthermore, they have established that in the guinea pig masses of spermatozoa appear at the open fistula immediately after copulation. In the rabbit, spermatozoa appear at the fistula opening in from twenty minutes to two hours after copulation, thus demonstrating that the operation (fistula technique) does not interfere with normal functioning of horn. (It had been shown by earlier workers that the time interval for the rabbit is in the neighborhood of two hours. Quoted by Parker, 1931.)

The uterine fistula in the dog. Because of the many early failures to secure a permanent patent uterine fistula in the dog, the writer considers it advisable to briefly describe this procedure. Early attempts, in which the fistular ends of the uterine horns were sewed to the body wall as described by Amantea (1922), resulted in a closure of the opening several weeks after the operation. It was found that, simply by leaving 1 cm. of the horn clearly outside the surface of the skin, a permanent patent fistula opening could easily be established. The technique is as follows:

Under morphine-ether anesthesia, make a median longitudinal abdominal incision about 8 cm. long, between the second and third caudal pair of nipples. Locate the uterine horns by first securing the vagina beneath the bladder, following it anteriorly to the two horns of the uterus. Securely tie off the ostium of the oviduct, and free for about 2 cm. the apex of the uterine horn from the broad ligament and its accompanying blood vessels. Make a stab incision at the level of the second nipple at the border of rectus muscle, taking care to avoid mammary tissue, as this tissue seems to infect rather easily and heals slowly. Pass a long-nosed hemostat through the stab incision, grasp the apex of the uterine horn securely, and pull through the stab incision. Take care that the uterine horn does not become twisted during this procedure. If a double uterine fistula is to be established, follow the same procedure on the other side, then close the median longitudinal incision, layer by layer. Attach the uterine ends to the skin by two or three linen subcutaneous stay sutures, allowing each stump to protrude from the skin by 1 to 1.5 cm. If the operator is careful not to crush the uterine stump while pulling it through the stab opening, no sloughing will occur. Allow to heal for two weeks, after which time the dog may be used for these experiments, or for uterine motility studies by the balloon method.

It is not advisable to carry out uterine surgery during the oestrous period, or for at least a month following parturition, as unnecessary bleeding will otherwise be encountered. This technique is not to be confused with the Reynold's uterine fistula technique in the rabbit (1929), which employs the vaginal end of the uterus, at the cervix.

Ascent of spermatozoa in the dog. The experiments were carried out as follows: The dogs were led out to an open-air paddock, released from their ties and allowed to copulate in a natural manner. The time of beginning of copulation was taken with a stop-watch, and each of the different periods,

TABLE 1

EXPERIMENT AND DATE OF BREEDING	TIME OF ACTIVE COPULATION	ADVENT OF FIRST DROP OF SPERM AT FISTULA		END OF SPERM SUSPENSION AND BEGINNING OF WATERY SECRETION		VOLUME OF SPERM SUSPENSION (IVANOW'S "FIRST AND SECOND EJACULATE"?)	END OF ALL SECRETIONS DROPPING FROM FISTULA		VOLUME OF CLEAR FLUID (IVANOW'S "THIRD EJACULATE"?)	TOTAL DURATION TIME OF COPULATION	
	seconds	*minutes*	*seconds*	*minutes*	*seconds*	*cc.*	*minutes*	*seconds*	*cc.*	*minutes*	*seconds*
Female 9											
9-16-32	28		58								
9-17-32	25	1	45	4	10	3.0	12	5	21.2	13	30
Female 3											
9-21-32	40		65	3	10	2.5				15	10
9-22-32	25		50	4	30	2.7	15	5	18.9	18	20
9-23-32	25		45	3	15	3.2	16	30	25.0	19	20
9-24-32	25	1	15	3	05	3.0	17	15	16.3	21	45
9-25-32	20		55	5	00	6.0	14	17	19.7	18	25
Female 4											
9-25-32	20		60	3	30					10	
9-27-32	20	1	30	4	15	3.1	12	30	22.5	14	20
9-28-32	23	1	19	2	30	2.3	12	00	24.0	17	30
9-29-32	20	1	40	3	30	2.3	18	05	17.8	20	00
9-30-32	25	2	30	5	00	1.6	13	15	9.4	15	30

as noted in table 1, carefully recorded. Seminal fluids were collected from both fistulae into small beakers, which were taken to the laboratory, where their contents were examined under the microscope.

Table 1 illustrates the results of experiments on three females which came in heat this last fall. One male, a large collie (16.4 kilo), was used for service; of the other two males tried, one proved to be sterile, while the other was unwilling to service in the presence of persons.

The times recorded in table 1 are all taken from the moment the male "successfully mounted." It will be seen then, for example, in female 9

(9-16-32), the time of advent of sperm at the opening of the fistula is fifty-eight seconds after mounting or, more important, thirty seconds after ejaculation.

Any report on transport of spermatozoa in the dog must bear reference to the methodical experiments of Ivanow (1917), who determined the characteristics of ejaculation of the dog and showed that, instead of one ejaculate, there are essentially three distinct ejaculates, each having a different period of emission. Ivanow, after artificially exciting the male dog by masturbation, noted that

> erection is produced very quickly, the dog making repeated movements characteristic of coitus which ordinarily cease at the debut of ejaculation. At the debut of ejaculation, the dog agitates alternately the hind quarters. During ejaculation of the second portion and throughout the third portion, the extremities of the animal remain immobile, the tongue hangs from the mouth and the breathing is heavy. The expression during the third portion is characteristic of fatigue and languor.

Further, the duration of ejaculation of the first portion varies between 6 and 26 seconds, the second portion begins in from 8 to 26 seconds and lasts from 10 to 44 seconds, while the third portion, which demands the greatest length of time, takes from 4 to 16 minutes, or even longer. The volume of first and second secretions is close to 1 or 2 cc. and contains the greatest number of spermatozoa ejaculated during the secretion of the second portion; the volume of the third secretion varies between 14 and 30 cc., constituting the principal liquid portion of the semen.

Thus it may be seen from Ivanow's work that in dealing with ejaculation of the dog we are confronted with a complex reaction. Efforts were made to differentiate between the different portions of the ejaculate arriving at the fistula openings of the females used in this study. They are at best only rough estimates.

As the table illustrates, active copulation on the part of the male took place generally for about 25 seconds. The term "time of ejaculation" is used advisedly; in this the writer has followed Ivanow's description of the nervous reaction of the artificially excited male. That the same reaction holds true for the male in coitus is evidenced by the fact that, almost invariably, 25 seconds after the male has "successfully mounted," he will give three to ten especially intense thrusts, his fore legs then falling to one side of the female, though still attached. This reaction the author considers the completion of the "active ejaculation." The other portions probably dribble into the vagina. Ordinarily within 25 to 50 seconds, a sperm suspension will be issuing from one or both fistulae of the uterine horns. At this point, strenuous abdominal muscle straining by the female commences. After each straining, semen will be forced out the fistula in small streams, often to a distance of 20 to 25 cm. (as in forcing fluid from a tuberculin syringe). From about 3 to 5 minutes after ejaculation, the

cloudy sperm suspension coming from the fistula seems to be replaced by a water-clear solution. This the author believes to be the first of Ivanow's "third portion" to arrive at the fistula. This clear fluid continues to flow until about three minutes before the dogs are apart. At this time the female makes the first determined attempts to separate herself from the male. At from ten to twenty minutes after the start of copulation, the dogs will usually be apart. When the female pulls away from the male, about 10 cc. of cloudy sperm suspension will flow from the vagina.

As the table illustrates, the volume of first cloudy portion (presumably Ivanow's first and second portions) is about 2 to 3 cc., and the volume of the third portion about 14 to 26 cc. When examined under the microscope, the cloudy suspension obtained first will be seen to contain from 10 to 100 times as many spermatozoa per cubic centimeter as the water-clear secretion. These figures closely resemble Ivanow's data.

Discussion. The possibilities of several mechanisms acting to carry sperm into the uterus from the vagina has been so fully discussed by Hartman (1932, pp. 694–699) that little can be added. Needless to say, the writer has little evidence that Amantea's (1924) explanation of the rapid ingress of sperm into the uterus, namely, direct ejaculation, does not hold true for the dog. Unpublished experiments by Templeton and the writer would indicate that the vagina of the dog elicits powerful contractions normally and that these contractions are adequate to force spermatozoa into the uterus, en masse, if the cervix is open.

Hartman (1932, loc. cit., p. 609) maintains that in the rat, spermatozoa are carried like inert particles through the entire length of the uterus. It would seem that the same description might well be given for the dog, when the rather exact data listed in table 1 are considered. Hartman considers that the enormous accumulation of fluid in the uteri of the rat offers a favorable medium for the mixing of the spermatozoa and presumably adds to the bulk for propulsion up the tract to the oviducts. The writer was not impressed with the amount of fluid found in the uterine horns of the dogs used in these experiments and it is his belief that they contain scarcely more fluid during oestrus than the relatively dry rabbit uterine horns. The total volume of fluid collected from the horns agrees quite well with the volumes of ejaculate recorded by Ivanow.

In considering factors responsible for the rapid transport of spermatozoa through the uterine horns of the dog, the two most probable appear to be antiperistaltic movements of the uterus and the alternately increased intra-abdominal pressure brought on by the straining of the female during "orgasm" as witnessed by the writer. Each straining was followed by the appearance of more sperm suspension at the fistula opening.

Certainly the ciliary activity observed by Parker for the turtle and pigeon plays no rôle in the case of the dog. Even if cilia are present throughout

the entire length of the uterus, their beat is too slow to account for the "almost catapultic hurtling" of the sperm fluid up the uterus in this species. The author considers the process in all probability to be something as follows: Following ejaculation into the vagina in the region of the cervix, the cervix relaxes, preceding an anti-peristaltic wave of contraction starting in the vagina; this wave then passes successively over the cervix, body of uterus, clear up to the apex of the horns. During the straining movements of the female, the cervix may be closed, thus affording an opportunity for thorough mixing of sperm fluids and their passage to a point of least resistance, i.e., out the fistula opening or, normally, into the oviduct.

Parker (loc. cit., p. 412) has given much the same interpretation to Hartman and Ball's findings for the rat. In the rabbit the wave spreads only to the cervix, the sperm being deposited in the body of the uterus. The rest of the journey to the oviduct is made without the aid of anti-peristaltic waves. In the rat the waves "may spread, however, presumably as waves of anti-peristalsis, up the ducts even to their ovarian ends."

In two experiments, the writer introduced into the vagina of two dogs (no. 9 and no. 4) during oestrus, 10 cc. of sperm suspension obtained from the uterine fistula of another dog during copulation. Samples were taken by means of a small glass tube from the openings of the uterine fistula at 10-minute intervals. Up to one hour and forty minutes no sperm could be demonstrated to have arrived at the openings of the fistula. This the writer considers as supportive evidence that there is some nervous reaction on the part of the female dog during "orgasm" which aids materially in the propulsion of sperm suspensions up the uterine horns.

There still remains the problem of how much time must elapse before the spermatozoa enter and pass up the oviducts where fertilization of the egg can take place. Bischoff was quite firmly convinced and, in fact, drew the conclusion that it was only in the course of the following hours that spermatozoa enter the oviduct. It seems to the writer, after viewing the force with which sperm suspensions are ejected from the uterine fistula, that unless some mechanism is demonstrated at the mouth of the oviduct which would impede the progress of a mass of sperm being propelled up the uterus, we must believe that some spermatozoa do actually enter the oviduct at this time. In fact, we do have one recorded observation (Whitney) that spermatozoa do actually enter the oviducts and proceed as far as the ovarian bursa during the copulation period of the dog.

Finally, one should proceed with caution in making generalizations relative to the transport of spermatozoa in other species. There are those who would postulate from these data on the transport of spermatozoa in the dog, and from those of Hartman and Ball for the rat, that in other species, including man, spermatozoa appear at the oviduct a few seconds

after orgasm. In the human, this matter has an immediate practical bearing on the development of useful and effective contraceptive factors.

In animal breeding, knowledge of the actual time required for the transport of spermatozoa (and time of ovulation) might well alter breeding practices now in vogue. It is certain that application of artificial-insemination technique in domestic animals introduces a new problem, i.e., do spermatozoa injected artificially into the vagina take a longer time to reach their destination at the oviduct than spermatozoa entering the vagina in normal coitus? The solution of these problems is the goal of investigations now being carried on in this laboratory.

SUMMARY

It has been demonstrated by means of uterine fistula technique, that in the dog, spermatozoa arrive at the oviducts within twenty-five seconds after ejaculation by the male. It seems very probable that these spermatozoa are transported, en masse, by anti-peristaltic waves passing up the uterus, and that ciliary activity plays little or no rôle in the transport of spermatozoa in this species.

REFERENCES

AMANTEA, G. 1922. Annali di Ostetrica e Ginecologia, xliv, 344.
1924. Rassegna di Studi Sessuali, iv, 306.
BISCHOFF, T. L. W. 1845. Entwicklungsgeschichte des Hunde-Eis. Friedrich Vieweg u. Sohn, Brannschweig.
FLOREY, H. AND A. WALTON. 1932. Journ. Physiol., lxxiv, 5.
HARTMAN, C. G. AND J. BALL. 1930. Proc. Soc. Exp. Biol. and Med., xxviii, 312.
HARTMAN, C. G. 1932. Sex and internal secretions, E. Allen, Editor. Williams & Wilkins, Baltimore, 1932, pp. 647–733.
IVANOW, E. 1917. Compt. Rend. Soc. de Biol., lxxx, 517.
PARKER, G. H. 1931. Phil. Trans. Roy. Soc., B, ccxix, 381.
REYNOLDS, S. R. M. 1930. This Journal, xcii, 420.
WHITNEY, L. F. 1927. The mating cycle of the dog. Chase Mag.

HYPERVITAMINOSIS D AND BLOOD PRESSURE IN DOGS[1]

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Received for publication May 1, 1933

The recent reports on arteriosclerotic changes produced in rats (1) (2) (3), cats and rabbits (4) (5) and dogs (6), by feeding them for long periods with high doses of vitamin D, have led us to find out whether or not the arteriosclerotic changes were accompanied by variations in blood pressure.

METHOD. The data given here have been obtained from twenty dogs, of which 13 were fed moderately high doses of vitamin D and 7 acted as controls. Since Livingstone, Andrews and Adams (7), as well as Brooks and Luckhardt (8) have pointed out that indirect determinations of blood pressure are subject to serious errors, we have resorted to the more painstaking but reliable direct graphic method. Under ether anesthesia and aseptic precautions, the right carotid artery was cannulated with a glass T-shaped cannula. The cannula was connected through rubber tubes to a U-shaped mercury manometer which was made to register blood pressure through an aluminium pointer fixed to a float in the usual manner. A kymograph tracing of blood pressure was taken for ten minutes, then 1 cc. of a 1:1000 solution of adrenalin hydrochloride (Parke, Davis & Co.) was injected into the left external jugular vein and its effect on blood pressure was recorded. After the blood-pressure curve has returned to normal, the cannula was removed, the carotid artery ligated with chromic cat gut, and the wound carefully closed and bandaged. Each dog was then placed in a separate cage and kept on a mixed diet. In addition to its regular ration, each dog received every other day 0.20 gram of calcium chloride per kilo body weight, mixed with the food. Moderately high doses of vitamin D were administered in the form of "vigantol." Vigantol is a proprietary name given by Bayer, Meister & Lucius, Leverkusen, Germany, and E. Merck, Darmstadt, Germany, to a standardized preparation of irradiated ergosterol of which 1 cc., which corresponds to 25 drops, represents 50 clinical units. This preparation was given to the dogs by stomach tube, followed by 10 cc. of olive oil to wash into the stomach any drops of vigantol that might have remained on the walls of the rubber tube.

Eleven out of the thirteen vitamin D fed dogs were given 9 drops of

[1] A preliminary report was read at the 14th International Physiological Congress in Rome, 1932.

vigantol per kilo body weight, while the other two dogs had 7 drops per kilo body weight daily. The feeding of vigantol was done regularly early in the morning on empty stomachs.

An exactly similar procedure was followed in handling the control dogs, with the exception that they were given 10 cc. of olive oil by stomach tube with no vigantol. To prevent any contamination with vigantol, the stomach tubes used in the controls were never used for administering vigantol to the animals.

At the end of the experimental feeding period consisting of 15 days for 2 animals and 25 days for the rest, the dogs were again anesthetised and their blood pressures recorded by cannulating the left carotid artery and then the right carotid artery in case it remained patent, and connecting it to the mercury manometer. After obtaining a ten-minute blood pressure tracing, the adrenalin effect was tested again in exactly the same manner as it was done previous to the feeding of vigantol. The dogs were then killed by hemorrhage, post-mortem examinations made and sections of the various tissues prepared.

RESULTS. Ten out of the thirteen dogs that were treated with vitamin D remained during the experimental period in excellent health, gaining in weight just as much as the six healthy controls. Three dogs that were fed vigantol and one control contracted lung infection in the course of the experiment. Accordingly for convenience of description of the results the animals were classified into two groups as shown in table 1. The first group includes the vigantol fed animals, the second group the control animals. At the end of the experimental period blood pressure on the ten healthy treated animals showed a markedly consistent rise over the pre-experimental level, ranging between 14 and 90 mm. Hg. In all other animals with the exception of one control, no. 5, a very slight rise of 4 and 6 mm. Hg or a considerable fall in blood pressure was noted instead. Dog 5 showed a rise of 12 mm. Hg, which was smaller than the general rise observed in the healthy treated dogs. Four out of the six controls showed a fall in blood pressure ranging between 10 and 33 mm. Hg. The general average for the rise in blood pressure in the 10 healthy treated animals was 32 mm. Hg and the average condition for the 6 healthy controls was a fall of 9 mm. Hg.

The characteristic response of the blood pressure level to the adrenalin injections was modified in practically all the ten dogs that were fed vigantol and remained in good health throughout the experiment. The modified response consisted of a lesser rise in blood pressure followed by a subsequent deeper fall, i.e., the average rise being 51 mm. Hg before the feeding and 38.9 after the feeding, and the average fall being 40 mm. Hg at the beginning of the experiment and 56 at the end. The rise brought about a blood-pressure level that was nearly attained by means of adrena-

lin before the administration of vitamin D, and the subsequent fall in blood pressure reached a level that was almost the same as that noted before vigantol was given.

An exception to this general behavior was offered by dog 14 in which the blood-pressure level attained under adrenalin was much greater after the

TABLE 1

Showing the various data obtained on blood pressure of the two groups of animals

DOGS FED VITAMIN D	DURATION OF EXPERIMENTAL PERIOD	NUMBER OF DROPS OF VIGANTOL PER DAY PER KILO BODY WEIGHT	B/P VALUE AT THE BEGINNING OF THE EXPERIMENTAL PERIOD	B/P VALUE AT THE END OF THE EXPERIMENTAL PERIOD	DEVIATION IN B/P VALUES	EFFECT OF ADRENALIN ON B/P BEFORE FEEDING VITAMIN D				EFFECT OF ADRENALIN ON B/P AFTER FEEDING VITAMIN D			
						Maximum rise	Duration of rise	Maximal fall	Duration of fall	Maximal rise	Duration of rise	Maximal fall	Duration of fall
no.			*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*seconds*	*mm.* Hg	*seconds*	*mm.* Hg	*seconds*	*mm.* Hg	*seconds*
1	15	9	110	155	+45	50	128	10	120	29	70	70	200
8	15	9	134	167	+33	54	114	20	180	49	120		
10	25	7	125	193	+68	84	96	40	110	29	10	70	240
14	25	9	146	170	+24	34	120	30	14	90	100	60	180
2	25	9	134	156	+22	22	40	40	20	24	14	16	20
2a	25	9	110	130	+20	28	130	40	370	30	88	40	170
11a	25	9	140	160	+20	64	42	70	400	42	40	94	800
12a	25	9	130	220	+90	68	320	40	490	26	126	54	144
13a	25	9	130	160	+30	54	46	44	380	18	58	80	520
15a	25	9	176	190	+14	54	130	56	240	36	186	43	280
Averages.....			133.5	170.1	+36.6	51.2	116.6	39	232.4	37.3	81.2	58.5	283.3
CONTROLS													
no.													
4	25	0	150	154	+4	82	100	50	220	62	54	22	46
5	25	0	134	146	+12	98	160	30	120	122	100	40	60
7	25	0	133	100	−33	55	80	32	240	68	64	20	140
1a	25	0	104	90	−14	86	180	28	230	86	200	27	400
7a	25	0	138	124	−14	56	200	60	380	54	148	46	320
14a	25	0	140	130	−10	70	92	66	500	60	119	52	
Averages.....			133.1	124.6	−9	74.5	135.3	44	281.6	75.3	114	34.5	193.2

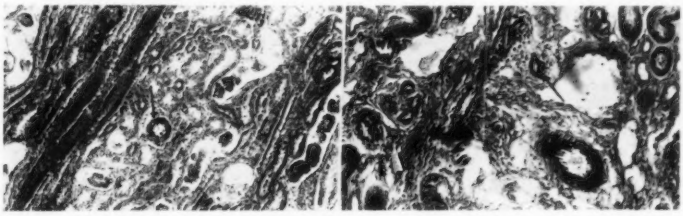
experimental period than before. The response to adrenalin did not undergo the same change in the control animals. In the animals that were fed vitamin D and that contracted disease the initial adrenalin rise was also limited, but the delayed adrenalin fall was slight.

In addition, the duration of the adrenalin rise in blood pressure in the

healthy animals before feeding vigantol was decreased from an average of 116.6 seconds to that of 81.2 seconds at the end of the feeding period, while the duration of the adrenalin subsequent fall in blood pressure was increased from an average duration of 232.4 seconds to 283.3 seconds. In the control group of anmals the average adrenalin rise was also shortened in duration from that of 135.3 seconds at the beginning of the experimental period to 114 seconds at the end of the period, but the average duration of the adrenalin fall in blood pressure was markedly decreased from 281 seconds to 193.2 seconds. These various data are reproduced in table 1.

Pathological findings. Post-mortem macroscopic examination has revealed pneumonic red and gray hepatisation of the lungs, in some of the dogs.

On microscopic examination, the kidneys and the skeletal muscles of the healthy dogs that were fed vigantol have shown marked changes in the arterioles, consisting of hypertrophy of the media with no calcifica-



A B

Fig. 1. Section A showing the normal renal arterioles of dog 1a. Section B showing the hypertrophied renal arterioles of the vigantol fed dog 12a. The arrows point to characteristic vessels.

tion. Two out of three of the sick vigantol fed animals have also shown the same changes, while a third dog of this group had normal arterioles. On the other hand, in five out of the six controls, the arterioles of the kidney as well as of the other structures examined appeared unaffected. In the sixth control, dog 4, distinct arteriolar hypertrophy was noted. The contrast between the normal renal arterioles in the controls and the hypertrophied renal arterioles in the vigantol treated dogs is illustrated in figure 1.

Discussion. The consistent rise in blood pressure observed in the large majority of the healthy animals that were fed high doses of vitamin D might suggest the raised viscosity of the blood by the increased concentration of the serum calcium, as the causative factor in raising blood pressure. But Jones, Rapoport and Hodes (9) have reported that there exists no increased blood concentration nor signs of increased blood viscosity accompanying the hypercalcemia that was induced by feeding excessive

doses of irradiated ergosterol to thyroparathyroidectomized dogs. The possibility of an increased heart action as a cause of the rise in blood pressure seems improbable on account of the fact that our pathological studies did not reveal any signs of cardiac hypertrophy. The factor which might have given rise to an increased blood pressure is an increased peripheral resistance produced by arterial changes. As our pathological studies have definitely confirmed the existence of changes in the vessels consisting of hypertrophy of the muscular layer of the arterioles in the healthy ten treated dogs and its absence in the rest of the animals, we feel justified to conclude that the changes in the vessels are mainly responsible for the changes in blood pressure. On the other hand our physiological findings lead us to think that the arterioles of these animals have not been hardened by calcification and that they are still capable of undergoing vasodilatation such as was observed under the influence of adrenalin subsequent to the initial rise. Our pathological findings seem to confirm this assertation, as no calcification could be demonstrated in the hypertrophied arterioles. Furthermore, since the preliminary adrenalin rise in blood pressure reaches a level which is approximately the same as the one obtained under normal conditions, and the delayed vasodilatation gives rise to a fall in blood pressure reaching a level that is also attained with no vitamin D treatment, we are forced to conclude that vitamin D affects blood pressure in much the same way as adrenalin and ephedrine, namely, by stimulation of the vasoconstrictor mechanism, which in this case is continuous, resulting in a prolonged vasoconstriction and eventually under high doses would give rise to hypertrophy of the vascular musculature in the arterioles, as we have found in our animals. In such animals, the adrenalin effect is chiefly vasodilatation because the blood vessels of these animals are already in a state of vasoconstriction, which in some cases is of maximal intensity.

The criticism of a possible effect of substances other than vitamin D, that might be present in vigantol, is quite in place and to meet this criticism we are in the process of repeating the experiment with other vitamin D preparations.

CONCLUSIONS

1. Vitamin D in the form of vigantol was fed to dogs in moderate doses for periods of 15 and 25 days and produced a marked rise in blood pressure, along with a modified response of blood pressure to adrenalin.

2. The underlying cause of the rise in blood pressure is referred to the hypertrophy of the media of the arterioles which was found in the vitamin-fed animals.

3. Vitamin D has probably some influence on the maintenance of normal blood pressure, its action when given in slight excess being adrenalin-like in nature.

The writer wishes to acknowledge his thanks to Dr. Philip Sahyoun for the pathological studies of the tissues and to Mr. E. Goldberg of the Bayer Meister Lucius Co. for the generous supply of vigantol used in this work.

REFERENCES

(1) Sweeney, M. and E. Smith. This Journal, 1930, xcv, 620.
(2) Hoyle, J. C. Journ. Pharm. Exp. Therap., 1930, xxxviii, 271.
(3) Herzenberg, H. Beitr. Path. Anat. u. z. Allg. Path., 1929, lxxxii, 27.
(4) Schiff, A. Virchow's Arch., 1930, cclxxviii, 62.
(5) Vanderveer, H. L. Arch. Path., 1931, xii, 941.
(6) Hess, A. F., H. R. Benjamin and J. Gross. Journ. Biol. Chem., 1931, xciv, 1.
(7) Livingstone, H. M., E. Andrews and W. E. Adams. This Journal 1931, xcvii, 588.
(8) Brooks, C. and A. B. Luckhardt. This Journal, 1916, xl, 49.
(9) Jones, J. H., M. Rapoport and H. L. Hodes. Journ. Biol. Chem., 1930, lxxxvi, 267.

PROTEIN METABOLISM IN PANCREATIC DIABETES

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Received for publication May 2, 1933

In severe pancreatic diabetes the constant relation between nitrogen and sugar in the urine has long been known and has indicated that only part of the energy of the protein catabolized was used by the animal. In this respect phlorhizin and pancreatic diabetes are similar. As might be expected, therefore, it was early found that phlorhizin increased the protein breakdown (Reilly, Nolan and Lusk, 1898). Confirming this in 1902, Rubner stated that the specific dynamic action of protein accounted for the raised respiratory metabolism known to follow injections of this drug.

Observations on pancreatic diabetes in man by Gephart, Aub, DuBois and Lusk (1917) showed that, if from the total metabolism was subtracted the specific dynamic action of the extra protein breakdown due to the disease, then all of their subjects had metabolisms well below the normal level. It should be pointed out that these patients were emaciated and therefore, if free from diabetes, would have had subnormal metabolisms. Bernstein and Falta (1916) also thought that extra protein breakdown would account for the raised gaseous metabolism of diabetes. Von Falkenhausen (1925) determined the nitrogen excretions of dogs before and after pancreatectomy. The respiratory metabolism was not determined, but calculations of the possible specific dynamic action of extra protein catabolism following the operation makes one suspect that there are other more important factors at work raising the metabolism in diabetes. Joslin (1923) has stated that "a consideration of these relations between urinary nitrogen and metabolism certainly tends to the conclusion that nitrogen metabolism in and of itself does not alone control the metabolism of the diabetic."

METHOD. To determine if the increased basal metabolism of diabetes in cats could be due to the specific dynamic action of protein, we had first to find what this specific dynamic action was in cats, and, secondly, how high the postabsorptive protein metabolism rose following pancreatectomy. Accurate determinations of nitrogen excretions over short periods of time were of first importance. Ordinarily a cat urinates only about once in 24 hours. Giving large quantities of water by stomach tube increases the frequency of urination only slightly and of course complicates any results, because nitrogenous products are excreted more rapidly than they are

produced during the first of the diuresis (see Heilner, 1906). A bladder fistula is also unsatisfactory. Therefore, catheterization seemed to be the only method suited to our needs. We used female cats because a rather large bore catheter may be slipped into the urethra quite easily.

The animal was placed on its back on the animal board, the head and front legs securely fastened and the hind legs held forward and upward by an assistant. After the vulva had been slightly distended by means of a nasal speculum, the urethral opening could be seen, and the catheter directed quickly and easily into place. This was then connected by way of a collecting flask to a water pump which developed a negative pressure equal to about 10 cm. of water. The suction was important in bringing about complete emptying of the bladder. Thereafter, 25 cc. of salt solution were forced into the bladder, withdrawn to the flask and the process repeated to make sure that no appreciable amounts of urine remained behind. The first sample of urine was of course discarded, but about four hours later the process was repeated and the sample saved for total nitrogen determinations. Occasionally the animal was catheterized a third time in order to check the results for the previous period. Usually the observations were repeated on other days. The Kjeldahl method was used in analyzing for nitrogen.

After frequent determinations of post-absorptive nitrogen excretions in normal animals had satisfied us concerning the accuracy of the method, we began the measurements of specific dynamic action of protein. At first we offered our post-absorptive animals fat-free minced muscle. When they refused to eat this we resorted to forced feeding with casein. This material was mixed with water to produce a paste and forced into the stomach through a small rubber tube. About 30 grams of casein were given for each determination of specific dynamic action. The measurement of the amount of protein burned at any particular time offers some difficulty because of the delay in nitrogen excretion (see Williams, Riche and Lusk, 1912). This factor is probably not of great importance, since we determined the metabolism over a six-hour period when the oxygen consumption was close to a plateau, and the nitrogen excretion had probably caught up with protein breakdown. If anything, a lag would make our figures for the specific dynamic effect too high, so that our conclusion that protein is not an essential factor in raising the metabolism of diabetes is even more impressive.

In the work on diabetes we have determined the nitrogen excretion of our cats before pancreatectomy, after the operation while receiving insulin, and lastly after the withdrawal of insulin.

The methods of measuring respiratory metabolism and of performing the pancreatectomy have been described in previous papers (Ring and Hampel, 1932, 1933).

RESULTS. The cats in this laboratory are fed large quantities of protein, and since all of our previous work was done on animals receiving such a diet, we have continued using it in this work. Of course, this means that even post-absorptive nitrogen excretion figures are high. They are never-

TABLE 1

Respiratory metabolism and nitrogen excretion in normal and diabetic cats

CAT	NORMAL		DIABETIC WITH INSULIN		DIABETIC WITHOUT INSULIN		INCREASED METABOLISM ACCOUNTED FOR BY SPECIFIC DYNAMIC ACTION OF PROTEIN, USING METABOLISM OF DIABETIC CATS RECEIVING INSULIN FOR COMPARISON
	Basal metabolism	Nitrogen excretion	Basal metabolism	Nitrogen excretion	Basal metabolism	Nitrogen excretion	
	calories per hour	*mgm. per hour*	*calories per hour*	*mgm. per hour*	*calories per hour*	*mgm. per hour*	*per cent*
525	4.66 4.79	31.2 30.5*	4.32 4.38*	110.0 89.7*	6.74 6.76	111.0	3.0
527	4.67	44.0 44.0*	4.75	77.8	5.84	40.4	None
528	5.06 5.00	69.8 67.0* 68.2*	5.44 5.38 4.55* 4.58	136.1 88.8*	5.40† 5.52 5.85*†	138.5† 86.2*†	None
530	4.97 5.31	47.3	4.07	82.0	5.63 5.50	91.2	12.0
535	5.01	56.2			5.50† 5.68	55.2†	
536	5.83	58.1			8.52 7.35* 7.03	125.0 49.0*	
539	5.84	88.0	6.19 6.30	78.5 81.6	7.42 7.75	154.0	95.0

* Determined several days later than figures above.

† These animals lost weight so that total metabolism does not appear to have greatly increased. Comparing metabolisms per square meter of surface, the increase is 30 per cent or more.

theless surprisingly constant. For instance, at intervals of about four days, cat 528 excreted per hour, 69.8 mgm., 67.0 mgm., and 68.2 mgm.; cat 525, 31.2 mgm. and 30.5 mgm.; and cat 527, 44 and 44 mgm. Keeping the animals without food for 24 instead of 18 hours did not affect the figures for

nitrogen excretion. If one calculates these results in terms of body weight or surface area, he finds that they are similar to those found in the paper by Fiske and Sokhey (1925).

After pancreatectomy (table 1), the nitrogen excretion in most cases was raised while the animals were receiving insulin, even though their respiratory metabolisms were normal.

The withdrawal of insulin produced the usual rise in metabolism, but only in one cat, 539, did it cause any considerable increase in nitrogen excretion.

The specific dynamic action of casein in two normal cats was found to represent 72 per cent and 74 per cent of the extra protein catabolism of the period. This is close to the figure of 76.4 per cent determined on man by Aub and DuBois (1917). It is higher than the results of Williams, Riche and Lusk (1912) on dogs, namely, 45 per cent.

Chambers and Lusk (1930) state that "the specific dynamic action appears to be the same in the normal as in the diabetic dog." This agrees with a single observation we made, i.e., that the specific dynamic action per gram of nitrogen excreted is unchanged by the diabetic state.

Discussion. It is interesting that diabetic animals receiving insulin should have normal basal metabolisms but that their post-absorptive nitrogen excretion should usually be increased. Wishart (1928) found a definite correlation between protein breakdown and basal metabolism, one so exact that it could be put into a formula. On the other hand, no such formula would be applicable to the results of Deuel, Sandiford, Sandiford and Boothby (1928) whose subjects lived on protein-free diets for long periods of time while their basal metabolisms and nitrogen excretions were carefully studied. Again, the observations of McClellan, Spencer and Falk (1931) showed that the basal metabolism of subjects living on an exclusive meat diet for a year was actually lower than on a mixed diet, though the post-absorptive nitrogen excretion was increased. Lastly, Völker (1931) and Wang, Hawks, Huddlestun, Wood and Smith (1930) found that the basal metabolism did not run parallel to the protein in the diet. Our observations on the increased post-absorptive protein catabolism in diabetic animals receiving insulin do not necessarily mean that a larger proportion of the bodily heat was being supplied by protein, for these animals were excreting considerable quantities of sugar. In some cases as much as half of the energy from protein was lost in this way.

There are two possible methods of evaluating the factor of specific dynamic action of protein in raising the metabolism in diabetes. One can use as the basis for comparison the nitrogen excretion of the normal animal, and subtract this from the nitrogen excretion of that same animal while diabetic and not receiving insulin. The extra protein burned as represented by the increased nitrogen excretion is multiplied by the appropriate

figure to determine its specific dynamic action. We can then see what proportion of the increased metabolism of diabetes is due to this factor.

As an example, let us take the figures for cat 525. The normal nitrogen excretion averaged 30.8 mgm. and in the diabetic state without insulin increased to 111.0 mgm. The difference of 80.2 mgm. represents the extra protein metabolism following pancreatectomy; 80.2 mgm. multiplied by 0.02651 (see Lusk, 1928) gives the heat coming from protein, 2.12 calories. The specific dynamic action is 73 per cent of 2.12 calories (see text) or 1.55 calories. The increase in basal metabolism brought on by diabetes equals 6.75 minus 4.72, 2.03 calories. The figure 1.55 calories is 76 per cent of 2.03 calories. Therefore, the specific dynamic action of protein represents 76 per cent of the total increase in basal metabolic rate, if we use the normal metabolism as the basis for the calculation.

The other and perhaps more satisfactory standard for comparison is the diabetic animal receiving insulin and showing a normal basal metabolism. Table 1 shows our results calculated to this standard in the last column. It will be seen that in only one out of five animals did protein play an important part in raising the metabolism. In this cat, 539, the measurements were made just a short time after the operation. If we had waited a week longer, it is probable that the protein breakdown would have been lowered without a corresponding change in respiratory metabolism. In cat 536, the first determination of nitrogen excretion when the animal was severely diabetic showed 125 mgm. per hour. A second one four days later was 49 mgm., though the respiratory metabolism was still well above normal. Similar figures for cat 528 were 138.5 and 86.2. It looks as if the nitrogen excretion tended to fall at a time when the respiratory metabolism was still high.

A comparison of the cats in the normal state with their condition in diabetes shows that in three animals the protein metabolism is not increased. In four others, protein could account for the raised metabolism of diabetes. However, since other factors are apparently introduced by pancreatectomy, it would seem more logical to compare the conditions in operated animals receiving insulin with their state when insulin was withdrawn. At any rate, one is justified in saying that there is a factor other than protein which may cause the increased metabolism of diabetes and that this other factor is usually of more importance than the specific dynamic action of protein.

CONCLUSIONS

1. Nitrogen excretion is usually increased in diabetes (see table 1).

2. There must be some factor aside from the specific dynamic action of protein to account for the increased metabolism of diabetes in cats (see table 1).

REFERENCES

AUB, J. C. AND E. F. DUBOIS. 1917. Arch. Int. Med., xix, 840.

BERNSTEIN, S. AND W. FALTA. 1916. Deutsch. Arch. Klin. Med., cxxi, 95.

CHAMBERS, W. H. AND G. LUSK. 1930. Journ. Biol. Chem., lxxxv, 611.

DEUEL, H. J., I. SANDIFORD, K. SANDIFORD AND W. M. BOOTHBY. 1928. Ibid., lxxvi, 391.

FISKE, C. H. AND S. S. SOKHEY. 1925. Ibid., lxiii, 309.

GEPHART, F. C., J. C. AUB, E. F. DUBOIS AND G. LUSK. 1917. Arch. Int. Med., xix, 908.

HEILNER, E. 1906. Zeitschr. f. Biol., xlvii, 541.

JOSLIN, E. P. 1923. Carnegie Pub. 323, p. 122.

LUSK, G. 1928. Science of nutrition. W. B. Saunders Company, p. 68.

MCCLELLAN, W. S., H. J. SPENCER AND E. A. FALK. 1931. Journ. Biol. Chem., xciii, 419.

REILLY, F. H., F. W. NOLAN AND G. LUSK. 1898. This Journal, i, 395.

RING, G. C. AND C. W. HAMPEL. 1932. Ibid., cii, 460.
1933. Ibid., in press.

RUBNER, M. 1902. Energiegesetze, p. 370.

VÖLKER, H. 1931. Zeitschr. exp. Med., lxxv, 487.

VON FALKENHAUSEN, M. F. 1925. Arch. exp. Path. u. Pharm., cix, 249.

WANG, C. C., J. E. HAWKS, B. HUDDLESTUN, A. A. WOOD AND E. A. SMITH. 1930. Journ. Nutr., iii, 79.

WILLIAMS, H. B., J. A. RICHE AND G. LUSK. 1912. Journ. Biol. Chem., xii, 349.

WISHART, G. M. 1928. Journ. Physiol., lxv, 243.

THE LIVER AND THE RESPIRATORY METABOLISM OF PANCREATIC DIABETES

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Received for publication May 2, 1933

Mann and Magath (1923) have shown that the fall in blood sugar which follows hepatectomy is not prevented if the animal has been previously depancreatized. Total removal of the pancreas and partial removal of the liver were followed by either a slight increase or no change in blood sugar. The liver, therefore, is essential for the rise in blood sugar following pancreatectomy. This being the case, it seemed possible that the raised metabolism of diabetes might be due to extra work performed by the liver.

Furthermore, Hendrix and Sweet (1923) have brought evidence to show that dogs with an Eck fistula, which have been depancreatized, show practically no glucose in the urine if they are made to fast for several days. Is it possible that such animals would show a basal metabolism remaining at the normal level when insulin is not given? The blood supply to the liver of these animals would have to come primarily through the hepatic artery, being supplemented by one or two very small vessels from the diaphragm. It is apparent that the arterial blood going to the liver could not possibly carry sufficient oxygen for the 40 per cent increase in metabolism known to occur in simple diabetes. In a cat weighing 3 kgm., the liver would have to receive 40 cc. of arterial blood per minute. If the diameter of the hepatic artery is 1 mm., this would necessitate an average linear velocity of 70 cm. per second, i.e., more than twice the rate of flow in the carotid artery (see Wiggers, 1923). Figures given by Barcroft and Shore (1912) (see also Grab, Jannsen and Rein, 1930) for arterial circulation through the liver of the cat average 18 cc. per minute as compared with the 40 cc. necessary if in animals with an Eck fistula the liver accounts for the extra metabolism of diabetes. Therefore, a rise in the metabolism of diabetic cats with portal blood diverted into the inferior vena cava could only indicate that the liver is not responsible for this increase.

METHOD. Dragstedt (1931) has described a simple method for producing an Eck fistula in animals. Tying a loose ligature about the portal vein just behind the liver obstructs the circulation sufficiently so that small vessels leading into the inferior vena cava become enlarged. Two weeks

after the first operation the portal vein can be completely occluded and the collateral vessels will carry the blood.

It has been previously shown that the removal of the head of the pancreas does not increase the respiratory metabolism (Ring and Hampel, 1932). Therefore, in each cat, the head of the pancreas was removed and the portal was partially occluded. About a week and a half after this operation, the metabolism of the cats was measured. A second operation, consisting in the removal of the rest of the pancreas and the complete occlusion of the portal was performed, and final metabolism measurements were made.

For further testing of this question we decided to remove a large part of the liver rather than the whole organ, for in the latter case the animal would survive 24 hours at most and the results might be open to criticism. Mann and Magath (1922) have shown that removal of as much as 70 per cent of the liver in dogs may not cause death. Therefore, we extirpated about this amount in our diabetic animals by tying a mass ligature about the base of each lobe to be removed and then cutting as close to the ligature as possible.

Results. Table 1 shows the results obtained on cats with Eck fistula from which the pancreas was removed. Each had a normal metabolism while part of the pancreas was still present, but this increased with the complete removal of the pancreas even when the portal blood was diverted into the inferior vena cava instead of passing through the liver.

All cats from which from 50 to 70 per cent of the liver was removed showed the high metabolism of diabetes. Six such cats had a mean metabolism of 41.2 ± 0.5 calories per sq. meter per hour, and a respiratory quotient of 0.70 ± 0.004. The lowest metabolism obtained in this group was 36.7, which is well above the normal metabolism for cats (29.3 calories). These figures fit well with those obtained on simple diabetes in cats (Ring and Hampel, 1932).

Animals losing 35 per cent or more of their body weight have been excluded from this series. Inanition is known to lower metabolism, and even in these diabetic animals the metabolism under such conditions may fall within the normal range.

Discussion. *Specific dynamic action of protein in diabetes.* This paper adds support to the previous one showing that the specific dynamic action of protein is not essential to the increased metabolism of diabetes, for recent evidence points to the liver as the seat of the raised heat production after protein ingestion. Wilhelmj, Bollman and Mann (1928) state that "the intravenous injection of amino acids, alanine and glycocoll, into the hepatectomized animal fails to produce an elevation in the level of consumption of oxygen." Again, Dock (1931) has shown that "at least 85 per cent of the heat evolved as a result of a specific dynamic action of protein in chlore-

TABLE 1

Respiratory metabolism of diabetic cats with an Eck fistula

CAT NUMBER	DATE		R.Q.	CAL./SQ. METER/HR.
50	March 3	Portal partially occluded and head of pancreas removed		
	March 22		0.73	30.2
			0.73	29.5
	March 24	Rest of pancreas removed and portal completely occluded		
	March 26	Without insulin for 18 hours	0.65	41.4
			0.67	38.0
			0.70	38.8
	March 29	Without insulin for 18 hours	0.73	38.2
			0.73	38.5
44	February 16	Head of pancreas removed and portal partially occluded		
	February 25		0.77	30.9
			0.83	31.0
			0.75	31.2
	March 1	Rest of pancreas removed and portal completely occluded		
	March 3	Without insulin for 18 hours	0.65	41.0
			0.64	43.3
			0.64	38.8
	March 4	Without insulin for 18 hours	0.65	44.1
			0.72	43.2
	March 5	Without insulin for 18 hours	0.73	42.9
			0.71	44.2
			0.72	45.5
454	June 29	Depancreatized		
	July 9	Without insulin for 18 hours	0.77	35.8
			0.71	35.4
	July 12	Portal partially occluded		
	July 26	Without insulin for 48 hours	0.74	42.6
			0.77	43.9
	July 28	Without insulin for 18 hours	0.77	39.8
	August 1	Portal completely occluded—no more insulin given animal		
	August 3		0.72	48.3
			0.73	46.8
	August 5		0.76	42.9
			0.75	40.7
	August 8	Very severe acidosis	0.72	45.5
			0.70	47.3
	August 10	Less acidosis	0.72	48.8
			0.77	45.3

TABLE 1—*Concluded*

CAT NUMBER	DATE		R.Q.	CAL./SQ. METER/HR.
43	February 25	Head of pancreas removed and portal partially occluded		
	March 4		0.71	30.6
			0.73	29.8
	March 10	Rest of pancreas removed and portal completely occluded		
	March 12		0.70	43.0
			0.73	43.9
			0.70	44.1
421	March 30	Head of pancreas removed and portal partially occluded		
	April 6		0.70	28.4
			0.70	30.6
			0.71	30.2
	April 12	Rest of pancreas removed and portal completely occluded		
	April 14		0.70	42.2
			0.70	41.0
			0.71	41.2

TABLE 2

Respiratory metabolism of diabetic cats after partial hepatectomy

CAT NUMBER	DATE		R.Q.	CAL./SQ. METER/HR.
451	June 1	Pancreas and about one-half of liver removed		
	June 8	Without insulin for 18 hours	0.75	42.5
			0.73	43.6
	June 13	Without insulin for 48 hours	0.69	46.5
			0.68	48.1
446	May 31	Pancreas and about one-half of liver removed		
	June 6		0.69	40.9
			0.66	41.8
	June 9		0.75	36.3
			0.74	37.0
443	May 19	One-half of liver removed		
	May 31	Pancreas removed		
	June 4	Without insulin for 18 hours	0.66	39.3
			0.69	40.0

tone-anesthetized rats is liberated in the abdominal viscera." Nothhaas and Never (1930) believe that the presence of glycocoll in the blood in the liver but not in the muscles brings about an increase in the oxygen consumption. Only Rapport and Katz (1927) obtained contrary results when they found an increase of 55 per cent in the oxygen consumption in muscles perfused with blood containing glycin. On the whole, then, this work offers further evidence that protein catabolism is not requisite for the increased metabolism of diabetes.

Conversion of fats to carbohydrates in diabetes. One might suggest that the conversion of fat to carbohydrate would account for the raised basal metabolism of diabetes. Yet the exponents of this conversion believe that it takes place in the liver (see Macleod, 1928). Our failure to find a lowering of diabetic metabolism with partial hepatectomy or with the production of the Eck fistula does not support the view that the transformation of fat to carbohydrate accounts for the increased heat production.

CONCLUSION

1. Neither the production of an Eck fistula nor the removal of a large part of the liver prevents the rise in metabolism resulting from pancreatectomy (see table 1).

2. It seems unlikely that the liver is responsible for the increased metabolism of diabetes.

3. This work supports other evidence showing that the increased metabolism of diabetes is not due to the specific dynamic action of protein.

REFERENCES

BARCROFT, J. AND L. E. SHORE. 1912. Journ. Physiol., xlv, 296.
DOCK, W. 1931. This Journal, xcvii, 117.
DRAGSTEDT, L. R. 1931. Science, lxxiii, 315.
GRAB, W., S. JANSSEN AND H. REIN. 1930. Zeitschr. f. Biol., lxxxix, 324.
HENDRIX, B. M. AND J. E. SWEET. 1923. Journ. Biol. Chem., lv, 161.
MACLEOD, J. J. R. 1928. Fuel of life. Princeton Univ. Press, p. 90.
MANN, F. C. AND T. B. MAGATH. 1922. This Journal, lix, 485.
1923. Arch. Int. Med., xxxi, 797.
NOTHHAAS, R. AND H. E. NEVER. 1930. Pflüger's Arch., ccxxiv, 527.
RAPPORT, D. AND L. N. KATZ. 1927. This Journal, lxxx, 185.
RING, G. C. AND C. W. HAMPEL. 1932. Ibid., cii, 460.
WIGGERS, C. J. 1923. Circulation in health and disease. Lea & Febiger, p. 132.
WILHELMJ, C. M., J. L. BOLLMAN AND F. C. MANN. 1928. This Journal, lxxxvii, 497.

THE SEAT OF ACTION OF CYANIDE ON PULMONARY VENTILATION

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Received for publication May 19, 1933

In experiments demonstrating the predominant rôle of carotid sinus reflexes in the stimulation of pulmonary ventilation with cyanide salts, Heymans et al. (1931) claim to have demonstrated also that the only effect on pulmonary ventilation of cyanide at the bulbar centers is one of depression. Their procedure included the injection of cyanide into the common carotid, internal carotid, or vertebral artery, with the carotid sinuses denervated. It was believed that internal carotid injection, or common carotid injection with all the carotid branches occluded except the internal carotid, directs the injected dose with but slight dilution toward the medulla when the vertebral arteries are occluded in addition. Presumably in view of the fact that the vertebral arteries are of more importance in supplying the medulla than the internal carotid arteries, they cite also the case of an ineffective cyanide injection into a vertebral artery. In corroboration of negative or depressing effects that they report for such injections, they state that intravenous injections of cyanide after denervation of both sinuses and double vagotomy are without any stimulating action on respiration.

Apparently contrary to the Belgian worker's conclusions, Gesell (1928) obtained definite stimulations of respiration on injection of cyanide into the vertebral arteries of dogs in which common carotid injections themselves had but little effect except under conditions of occlusion such as mentioned above. Carotid respiratory reflexes arising from chemical stimuli had at that time not been demonstrated. It is therefore possible that in the processes of exposure of the various carotid branches the delicate innervation of the sinus region had been more or less damaged, or that in some peculiar manner the blood stream had been channeled past the chemically sensitive nerve terminations of the carotid body.

Further, Owen and Gesell (1931) obtained definite effects on respiration by injections of cyanide directly onto the floor of the fourth ventricle.

In view of these two apparent disagreements with Heyman's conclusions, an attempt has been made to determine the nature of both peripheral and central cyanide effects on respiration.

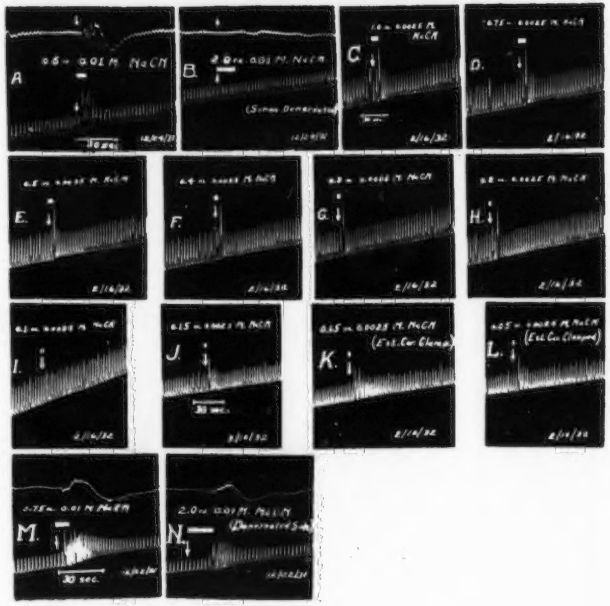
A
0.6 cc. 0.01 M. NaCN
B.
2.0 cc. 0.01 M. NaCN
C
D.
E.
F.
G.
H.
I.
J.
K.
(Ext. Car. Clamped)
L.
(Ext. Car. Clamped)
M.
30 sec.
N.

Fig. 1

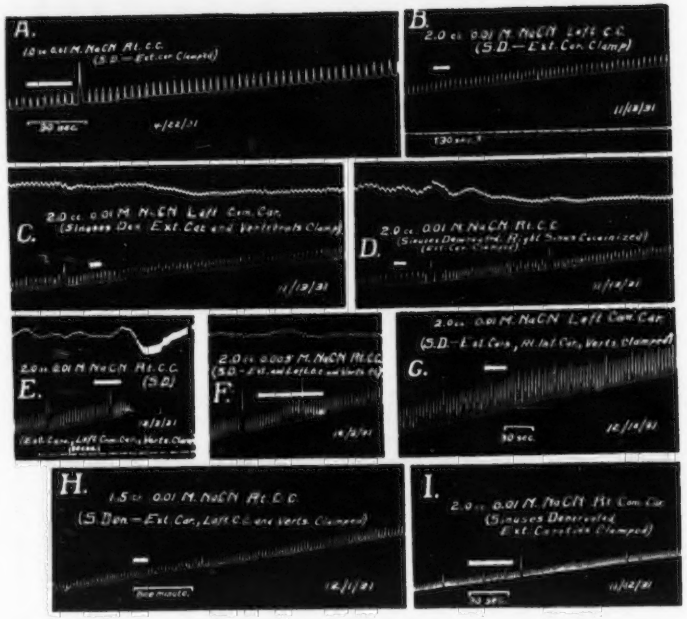
A.
10 cc. 0.01 M. NaCN Rt. C.C.
(S.D.—Ext. Car. Clamped)
30 sec.
B.
2.0 cc. 0.01 M. NaCN Left C.C.
(S.D.—Ext. Car. Clamp)
C.
2.0 cc. 0.01 M. NaCN Left Com. Car.
(Sinuses Den. Ext. Car. and Vertebrals Clamp)
D.
2.0 cc. 0.01 M. NaCN Rt. C.C.
E.
2.0 cc. 0.01 M. NaCN Rt. C.C.
(S.D.)
F.
G.
2.0 cc. 0.01 M. NaCN Left Com. Car.
(S.D.—Ext. Car., Rt. Int. Car., Verts. Clamped)
H.
1.5 cc. 0.01 M. NaCN Rt. C.C.
(S. Den.—Ext. Car., Left C.C. and Verts. Clamped)
I.
2.0 cc. 0.01 M. NaCN Rt. Com. Car.
(Sinuses Denerved.
Ext. Carotids Clamped)

Fig. 2

Blood pressure was recorded with a mercury manometer attached to a femoral artery, and respiration by means of a spirometer connected with a rebreathing circuit from which the animal breathed directly through a tracheal cannula in which dead space was reduced to a minimum. Dogs anesthetized with morphine and urethane (0.25 grain, and 1.0 gm. per kgm., respectively) or occasionally with amytal (0.065–0.077 gm. per kgm.) were used.

I. INJECTIONS OF SODIUM CYANIDE INTO THE COMMON CAROTID ARTERY.

A. *All innervation intact.* Doses approximating 0.5 cc. of 0.01 M. sodium cyanide in normal saline solution injected into patent common carotids whose sinuses were normally innervated, at rates varying between 1.0 cc. in 6 seconds and 1.0 cc. in 24 seconds, invariably resulted in marked and sudden increases in amplitude and, usually, rate of respiration, from 2 to 6 seconds after the beginning of the injection (fig. 1, A and M).

In an attempt to determine the minimum dose required for an effect, in each of 17 dogs of 15.0 to 26.5 kgm. body weight, a series of small injections was made into the normally innervated carotid. In ten of these animals the minimum was determined with all carotid branches patent, and in the remaining seven first with completely patent carotids, and then with the external carotids occluded so that the entire injection was directed through the internal carotid, the occipital artery, and the very small superior laryngeal branch coming off the common carotid near the sinus.

With the branches all open, the minimum doses required for a definite effect ranged from 0.0003 mgm. to 0.003 mgm. with an average of 0.001 mgm. per kgm. body weight. The solutions used were 0.01 M. or 0.0025 M. in 0.9 per cent NaCl. The effects of decreasing doses are shown for one animal in figure 1, C to I. With the external carotid clamped, the dose required was usually decreased to approximately one-third or one-half. Figure 1, J to L, shows the influence of such occlusion of the external carotid on the effectiveness of common carotid injections. It may be seen that for this animal, 0.05 cc. of 0.0025 M. solution injected with the external carotid clamped (L) caused very nearly the same effect as 0.15 cc. with the external carotid patent (J). Obviously enough, such an occlusion merely directs more of the dose past the nerve terminations in the sinus wall and carotid body. The carotid body is vascularly connected with the carotid system at the bifurcation, and appears to be largely, if not entirely, responsible for chemical reflex effects at the carotid zone (Dautrebande, 1931).

B. *Sinus denervated on the side of injection.* One carotid sinus was completely denervated by Hering's method (Hering, 1927); i.e., the common trunk of the carotid, the sinus, the internal carotid, and the external carotid, in the region of the bifurcation, were all stripped of their adventitia and separated completely from the glomus caroticum, occipital artery, and

tissue within the bifurcation, except where this tissue is vascularly connected in the angle of the fork; and the thus separated tissue within the fork tied with two ligatures and severed between them. The innervation of the sinus on the opposite side was not disturbed.

Doses of 1.0 or 2.0 cc. of 0.01 M. sodium cyanide in normal saline solution injected at rates of the order indicated in the previous section into the common carotid whose sinus was denervated were usually without effect, even though the corresponding external carotid was frequently ligatured in order to direct the dose more completely toward the Circle of Willis through the internal carotid (fig. 1, B). Occasionally, however, quite definite stimulations were obtained, the latent periods of which effects were longer than those resulting from injections into a normally innervated carotid (fig. 1, N). The extent to which these occasional stimulations were due to the passage of the injected dose across to the opposite intact sinus region through anastomoses, or to central stimulation, is not determined, hence any significance of such results is not stressed.

C. *Both carotid sinuses denervated.* Both sinuses were denervated in view of anastomoses mentioned above. A total of 109 injections was made in 32 different dogs. The animals varied in weight from 9.0 to 30.4 kgm. The rate of injection varied from 1.0 cc. in 29 seconds, to 1.0 cc. in 5.0 seconds, averaging 1.0 cc. in 11 seconds. Negative results only were obtained in 19 of the 32 animals. For 75 per cent of the negative results, the external carotid on the side of injection, or more frequently both external carotids were ligatured. From one to five injections per animal were made in this consistently negative group. The doses varied from 1.0 cc. to 3.0 cc. of 0.01 M. solution in normal saline, or from 25 to 75 times as large as the average minimal reflex dose given in a preceding section (section A). In one case the injection was made with both vertebral arteries occluded, and in another, with both vertebral and the opposite common carotid arteries occluded.

In the remaining 13 animals both negative and more or less definitely positive results were obtained. In three of these, redissection or local anesthetization of the sinus regions eliminated effects on respiration, so that they may be added to the 19 negative individuals. Doses of the ineffective injections after redissection or anesthetization were 1.0 cc. of 0.01 M. solution in two of the three cases, and 2.0 cc. in the other. One of the former two dogs persisted in being negative after occlusion of both vertebral and the opposite common carotid arteries.

Effects that had been obtained previous to investigating the completeness of the denervation were variable in these animals; viz., increased amplitude and rate, increased amplitude, increased amplitude and decreased rate, or increased rate. In one case, for two consecutive injections of the same dose on the same side, the first caused marked pauses initiated by a deeper breath, and the second caused a slight,

smooth increase in amplitude with a barely decreased rate. In another case, an injection on one side caused a decrease in rate whereas one on the other side caused an increase in amplitude and rate. The latent period until the first effect varied between 5 and 20 seconds.

On the reasonable assumption that these effects were of sinus reflex origin due to incomplete denervation, their variability is in accord with similarly varying results obtained by Danielopolu et al. (1930, 1931, 1932) on mechanical stimulation of the sinus, and which they attribute to the resultant effect of stimulating both inhibitory and excitatory fibers. The action of NaCN on the carotid sinus innervation in its normally intact condition is an invariable intense stimulation, thus demanding, if Danielopolu's two types of fibers exist and are capable of chemical stimulation by cyanide, that the excitatory type is predominantly affected. One is able to understand, nevertheless, that after an attempted denervation, when only a few of either or both types of fibers may be left intact as was possibly the case in the three experiments being considered, either or each consecutively might be predominantly affected.

Two experiments where effects and their latent periods resembled those before redissection or local anesthetization in the three experiments just considered, but where the denervation was not checked, will also be assigned to the negative group of animals. The total number of dogs, then, that may be considered as without response to injections into the common carotid, after sinus denervation, of doses 25 to 75 times as large as the average dose required to just excite the intact reflex mechanism, becomes 24 out of 32 dogs, or 75 per cent.

In six more animals some positive effects were obtained which cannot well be attributed to faulty denervation (fig. 2).

In the first of these to be considered, the first injection produced in 26 seconds a deeper breath followed by a maintained higher amplitude (fig. 2, A). The length of the latent period, in conjunction with the fact that sinus reflex effects are transient, suggests that this was a central effect. Two immediately following injections of the same dose had no effect on the persisting higher amplitude. In another of these six dogs, injections produced stimulations of rate in 20 to 30 seconds which were not eliminated by cocainization of the denervated sinus on the injection side (fig. 2, D), and which were intensified by occlusion of the vertebral arteries (fig. 2, B, C). In a third dog of the six being considered, a dose which was not effective previous to occlusion of the vertebral and opposite common carotid arteries caused, after their occlusion, an intense effect consisting of a primary increase in rate and secondary decrease in amplitude with considerable inspiratory tonus, ending in apnea (fig. 2, E). More carefully regulated doses in this dog resulted in slightly increased amplitude without depression (fig. 2, F). In a fourth dog of this group of six, a dose which produced no effect with the opposite internal carotid occluded, caused a very slight increase in amplitude and mean pulmonary volume when also the vertebral arteries were occluded (fig. 2, G). In a fifth of these six animals, a dose which had no effect previous to occlusion of the opposite common carotid and both vertebral arteries, was followed, after such occlusions, by a late gradual decrease and evening of amplitude, with a measurable rate increase (fig. 2, H). This is the only instance in this set of injections of what might have been an intracranially acting depression. It might have been spontaneous, in view of the fact that it was not apparent after a subsequent larger

injection. In the record for the last of this group of six dogs wherewith some more or less definitely positive effects were obtained, is another instance of a slight but definite late stimulation (fig. 2, I).

It is noteworthy that in all these six cases where effects cannot well be attributed to faulty sinus denervation, the external carotid arteries were occluded so that the entire stream of the common carotid past the origin of the superior thyroid and very small superior laryngeal arteries was diverted into the internal carotid artery toward the Circle of Willis. It should also be stressed that in several cases (12/3/31 and 12/10/31) femoral intravenous injections were made of doses such as used in the carotid, without effect, so that the results reported for carotid injections were not vagus reflexes.

Finally, there were two dogs, both anesthetized with sodium amytal, in which marked stimulations were consistently obtained from carotid artery injections past denervated sinuses, even after redissection of the sinuses, bathing them in ether, or painting them externally with cyanide without effect. This last procedure results in respiratory stimulation when the innervation is intact (Owen and Gesell, 1931). Injections made into the internal carotid of each of these animals also resulted in stimulations. Records from these experiments are shown in figure 3.

These two dogs may be considered in more detail. In the first, three injections of cyanide (1.0 cc. during 23 seconds, 1.0 cc. during 15 seconds, and 1.5 cc. during 14 seconds, of 0.01 M. solution) each resulted in definite increases in rate within 9, 8, and 7 seconds, respectively (fig. 3, A, B and C). Before the last of the three injections, the sinus on the side of the injection was redissected as a precaution against any possible intact nerve fibers. After it, the sinus on the opposite side was painted with 0.01 M. NaCN solution, externally, without effect. An injection was made into the internal carotid by insertion of the needle through the sinus wall some distance into that artery, with the same sort of stimulation resulting after a somewhat shorter latent period (fig. 3, D).

In the second of these two dogs, after painting each sinus externally with the cyanide solution without effect, 1.0 cc. of 0.01 M. solution in either common carotid produced increases in rate and amplitude (fig. 3, E and F). With respective injections occupying 10 seconds and 9 seconds, the latent periods were 4.5 and 8 seconds. After occluding the external carotids the effect was much more pronounced (fig. 3, G and H). Two subsequent injections of 2 cc. each, during 14 and 11 seconds, respectively, while the sinus on the injection side was painted with ether, produced the stimulation as before, after 8 and 7 seconds (fig. 3, I and J). These last injections were made at the end of a vigorous experiment, when the animal was less sensitive, so that the effects were less intense. Death occurred soon afterward. In this animal, also, injections into the internal carotid earlier in the experiment produced the stimulations (fig. 3, K).

Although 75 per cent of all the dogs in which the type of carotid injection being considered was made, can be classified as without intracranial response, the remaining 25 per cent, then, exhibited responses that appear

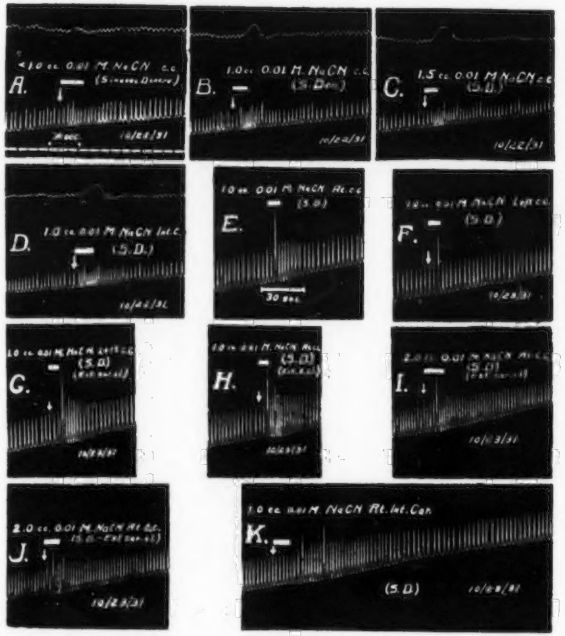

Fig. 3

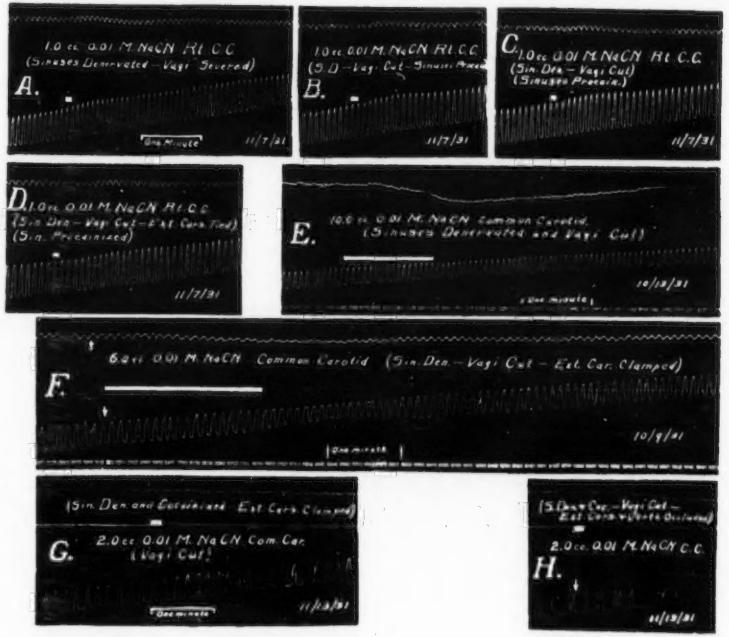

Fig. 4

definitely to be intracranial in origin. Some of these responses were marked. In but one case did the effect appear to be a depression. That effect might have been spontaneous, since it could not be repeated. The doses used where positive effects were obtained were 25 to 50 times as large as the average minimal reflex dose.

D. *Both sinuses denervated and both vago-sympathetic-depressor trunks rendered non-functional.* Hertzman and Gesell (1927) have obtained respiratory effects, though inconstant, from definitely localized vagal-ending stimulation with cyanide. Whether the endings stimulated were cardio-aortic, pulmonary, or abdominal, was, however, not determined by them. In view of evidence presented by Heymans and Heymans (1927) indicating that the pulmonary endings are not stimulated by ordinary anoxemia, one might expect that they would not be stimulated by cyanide. This expectation appears to be fulfilled in the work of Pagano (1900), who, using strong cyanide solutions in local injections concluded that the only vascular regions at which cyanide may set up a reflex respiratory effect are the left heart, aorta, and cephalic arteries. He obtained no effects on respiration from the action of cyanide in the pulmonary circulation, in systemic veins, or at the right side of the heart. If, then, cyanide has no reflex respiratory effect at the pulmonary vagal endings, it may not be argued that any of the positive effects discussed in the previous section were due to a return of the cyanide injected into the carotid back to the right side of the heart and thence to the pulmonary endings. The necessity may be entertained, therefore, that the blood carrying the injection would have had to return from the pulmonary bed to the cardio-aortic zone, where cyanide, according to evidence given, is capable of reflexly stimulating respiration in an inconstant manner (or to the abdominal region) before it could have given a vagal reflex effect. The latent periods of at least some of the effects being considered (fig. 3) were too short to allow for such an explanation.

Further, it would be difficult to understand why the effects could be increased or brought on by occlusion of the remaining cephalic arteries, especially where intravenous injections of the same dose had no effect (fig. 2; 12/3/31, 12/10/31), if the results were to be explained by vagal reflexes.

Finally, Gesell (1928) has shown that doses of cyanide of the order used for these injections are fixed in the tissues, and do not re-enter the circulation.

However, to exclude definitely the possibility that the effects were due to reflexes arising from vagal stimulation, it was deemed advisable to make several carotid injections with double vagotomy, or its equivalent, in addition to double sinus denervation. Such injections were made in eight dogs.

In two animals prevagotomy injections had not been made. In the first of these, with double sinus denervation and double vagotomy, two injections into the carotid of 1.0 cc. of 0.01 M. solution during 8 to 9 seconds were without effect. In the second,

prepared similarly except that the vagus trunks were rendered non-functional by means of an effective cold block system, two injections (1.0 cc. and 1.5 cc.) on the left side had no effect. On the right side, one 1.0 cc. injection during 5 seconds before cocainizing both the denervated sinuses (fig. 4, A), as well as two 1.0 cc. injections during 9 and 6 seconds, respectively, afterward (fig. 4, B and C), and then 1.0 cc. during 6 seconds after the external carotids were occluded (fig. 4, D), each caused a slight, but definite, smooth increase in amplitude. The latent periods of these positive effects ranged from 7 to 33 seconds. A femoral intravenous injection of 2.0 cc. at the beginning of the experiment was without effect, thus excluding the slight possibility that the carotid effects were due to generalized sensory stimulation in the pulmonary or systemic circulation. It may be seen that these effects are not of the abrupt type resulting from sinus reflexes.

Five of the dogs were of the group of 19 discussed above which were consistently negative to prevagotomy injections of the usual doses. Two of these five persisted in being consistently negative after vagotomy, one with the external carotid occluded on the side of the injection, the other with both external carotid and both vertebral arteries occluded. Doses as large as 2.0 cc. of 0.01 M. solution were used in each. Another of these five, which was negative to 0.5 cc. with the external carotids occluded prior to vagotomy, was also negative to four 1.0 cc. doses on the left side and one on the right after vagotomy; however, another injection on the right appeared to produce a very slight increase in rate and amplitude after 40 seconds. In this animal also 2.0 cc. injected into the femoral vein were without effect, even though before vagotomy.

In the fourth of these animals that were negative prior to vagotomy, the usual dose was not tried after vagotomy. The only injection made was that of 10.0 cc. during 78 seconds, which very large dose resulted in a very slight depression of respiratory amplitude and an increase in rate (fig. 4, E).

The last dog of this group of five had been negative to one 1.0 cc. dose injected into the carotid before vagotomy; after vagotomy as much as 2.0 cc. injected with the external carotids occluded was without effect. Larger doses (4.0 and 6.0 cc.) caused an increase in tonus of the muscles of inspiration resulting in less complete expirations, hence a slight decrease in amplitude, in one case followed by a slight increase in rate (fig. 4, F). This type of result, including a tendency toward pauses in the inspiratory position, was very similar to that of femoral intravenous injections in this dog under the same conditions of innervation (fig. 6, Q). The rates of injection were 1 to 4.5 seconds for each cubic centimeter for the femoral injections, 20 to 21.7 seconds per cubic centimeter for the carotid injections. It should be mentioned that whereas 4.0 cc. injected into the carotid produced the type of effect described (fig. 4, F), a dose of that size in the femoral vein had no definite effect, thus here also eliminating the possibility of an effect in the general circulation.

The eighth animal in which postvagotomy carotid injections with double sinus denervation were made, was one of the responsive dogs discussed in the previous section (fig. 2, B, C, D), where injections prior to vagotomy gave either increased rate or irregularity soon followed by increased rate of respiration. Both sinuses were cocainized and both external carotids occluded. A common carotid injection of 2.0 cc. caused a slight increase in rate and amplitude at 24 seconds (fig. 4, G). After both vertebrals had been occluded, rendering the centers considerably asphyxial, 2.0 cc. caused apnea in 55 seconds, which was interrupted once by a prolonged inspiration at 83 seconds, of the type accompanying respiratory failure (fig. 4, H).

It is indicated in this group of experiments that injections of 1.0 to 3.0 cc. of 0.01 M. NaCN into common carotids, past denervated sinuses, after

vagotomy as well as before, occasionally affect respiration. The effect, when obtained, is usually indicative of a stimulation. Regrouping all dogs used for injections into denervated carotids, both before and after vagotomy, doses 25 to 75 times as large as the average minimal reflex dose were ineffective in 24, or 71 per cent of the total of 34 animals.

It is supposed that the occasional stimulations are at least partially central in origin. The experiments themselves, however, do not exclude the possibility that there is some as yet unknown vascular reflexogenic zone, intracranially located, or that stimulations of superior regions of the brain by cyanide diverted through the internal carotid may result in an ultimate nervous stimulation of the respiratory nuclei in the brain stem.

The inconstancy and varied intensity of the stimulation among dogs would require that if such an intracranial vascular reflex zone as that suggested exist, it must either not be constantly present, or is not so located as to be consistently supplied by the internal carotid artery. A more probable explanation for the occasional occurrence of these stimulations, particularly in view of the fact that they were frequently brought on by occlusion of the vertebral arteries, is that the anastomosis of the internal carotid with the medullary circulation through the posterior communicating artery of the Circle of Willis is as a rule of small calibre in dogs. In cases where the variation is in the direction of a larger calibre, carotid injections may more readily descend toward the bulbar respiratory nuclei, even against the rich ascending supply through the spinal arteries. The latter of course remain patent while all accessible cephalic arteries are occluded. This probability will become more apparent after a consideration of the much more consistent effects obtained by vertebral intra-arterial injections.

II. Vertebral artery injections. A. *With both sinuses denervated.* A chemical injected into the vertebral artery is thrown into more constant and intimate relationship with the medullary centers than one injected into the carotid (with the occipital artery obstructed), because of the circuitous and variable connection of the latter with the medullary circulation. Vertebral injections are consequently of more importance in a study of the effect of any such chemical upon the bulbar respiratory "center." There is, however, a free vascular connection of the chemically sensitive carotid reflex zone with the veretebral artery (Winder, Owen, and Gesell, 1932), in view of which both sinuses were denervated or vascularly isolated and locally anesthetized previous to any of the vertebral injections now to be considered. The functional significance of this anastomosis will be considered in a later paper.

Injections were made with a fine needle directed either caudally or cephalically. A post-mortem examination of one of the dogs (30.4 kgm. body weight, 10/30/31) showed that on the left the distance from the subclavian artery to the point of injection was 2.5 to 3.2 cm. and on the

right 1.3 to 1.8 cm. The distance on either side from the aortic arch to the point of injection was approximately 6.3 cm. It is obvious, therefore, that in the case of the caudally directed injections, although some of an occasional dose might possibly have gone down along the subclavian artery, none could have reached the reflexogenic zones of the aorta. The rate of injection was smooth and not forced.

Forty-one injections were made in seventeen different dogs. For several injections one or more of the three cephalic arteries not used for the injection were occluded.

Twelve injections in six of the dogs were without effect.

In the first of these six dogs (16.0 kgm. body weight), 0.35 cc. of 0.01 M. solution injected during 3.5 seconds was without effect, with both carotid systems occluded. Following this, 0.5 cc. injected during 3 seconds produced a definite effect. In the second dog, of 24.5 kgm. body weight, where the carotid systems were completely occluded, 0.2 cc. during 3 seconds, 0.4 cc. during 7 seconds, and 0.5 cc. during 6 seconds, of 0.01 M. solution, were without effect. Following this, 1.0 cc. of 0.01 M. solution during 23 seconds caused a definite effect. After occluding the opposite vertebral artery (in addition to the two carotids), 0.75 cc. during 12 seconds caused a much more marked effect. In the third animal, of 14.2 kgm. body weight, the ineffective dose was 2.0 cc. of 0.00125 M. solution injected during 32 seconds. Previous to this, 1.5 and 2.0 cc. of 0.0025 M. solution each had caused definite effects. The vertebral artery injected into was the only patent cephalic artery in this animal. In the fourth animal, of 22.0 kgm. body weight, 1.0 cc. during 8 seconds, and 2.0 cc. during 10 seconds of 0.01 M. solution, were each without effect. After occluding the common carotid artery on the side of the vertebral injection, 3.0 cc. during 16 seconds caused a marked effect. In the fifth dog, 15.4 kgm. body weight, the ineffective injection of 1.0 cc. during 14 seconds of 0.005 M. solution was the only one made. It is reasonable to assume that a larger dose, at least after occluding one or more of the cephalic arteries not used for the injection, would have been as effective in this animal as in others considered. In the sixth animal, 1.0 cc. in 10 seconds, and 1.5 cc. during 12 seconds of 0.005 M. solution, 1.5 cc. during 20 seconds and 2.0 cc. during 13 seconds of 0.01 M. solution were all without effect. Earlier during the experiment, however, while the animal was in a better condition, even smaller doses caused marked effects. The cephalic arteries of this animal were all patent during the injections.

In only one animal, then, was a negative result *only* obtained. In this animal only one injection was made, and that was of comparatively small dosage (1.0 cc. of 0.005 M. solution). Ineffective doses ranged between 0.2 cc. of 0.01 M. solution injected during 3 seconds into the vertebral artery of a 24.5 kgm. dog with both carotids occluded, and 2.0 cc. of 0.01 M. solution injected during 10 seconds into a 22 kgm. dog with all cephalic arteries patent. In contrast with the number of negative effects, particularly when considering the fact that slightly larger doses, especially if injected while one or more of the cephalic arteries were occluded, *did* cause definite effects in the same animals, is the fact that in 16 of the 17 dogs, 29 injections were definitely effective. The dosage here ranged from 0.5 cc.

of 0.005 M. solution injected during 10 seconds into a 30.4 kgm. dog with all the cephalic arteries patent, to 3.0 cc. of 0.01 M. solution injected during 18 seconds into a 22 kgm. dog with the carotid occluded on the side of injection. The latter dose was excessive.

The interval between the beginning of the injection and the first respiratory effect, and the nature of the effect, varied with the size and condition of the animal as well as with the strength, quantity, and rate of injection of the dose used. The latent period range among 24 measurable values was 6 to 21 seconds, with an average of 14.5 seconds. The effects may be grouped:

1. Six effects in four of the animals were simple increases in rate and amplitude (in one case of amplitude alone), either returning to normal again with no depression, or with no more slight secondary depression than might be expected from the previous hyperventilation, or with considerable secondary depression or apnea (fig. 5, A, C, N).

2. Ten effects in five different animals were increases in rate followed by little or no secondary depression, or considerable depression chiefly in amplitude, or apnea (fig. 5, D, F, E).

3. In twelve cases among eight of the dogs, there was a late prolonged increase in amplitude as from a late stimulation. It followed a *primary* increase in rate and amplitude or in either rate or amplitude alone, and a secondary return to normal or depression in amplitude or rate or both, or a short apnea (fig. 5, G, H, I, J, K, L, O).

4. Only one of the 29 effects was a pure depression. This was obtained from a dog which was on a decline at the time of the injection, due to over-anesthetization with amytal (fig. 5, M).

It is very suggestive that occlusion of one or more cephalic arteries at the time of injection enhanced the effect, as might be expected from the presumption that such a procedure would cause the medulla to be supplied to a greater extent by blood from the vessel into which the injection was made (fig. 5, B and C; N and O).

B. *Both sinuses denervated and both vagus trunks rendered non-functional.* Mechanically it seems scarcely possible that injections into a vertebral artery might make their way against the blood stream as far back as the aortic reflexogenic zone. Considerations in connection with the carotid injections have shown that it is highly improbable that the injected doses might have returned into the venous circulation (Gesell, 1928). Considering that they might possibly have done so, they would have had no effect in the veins, right side of heart, or pulmonary circulation, according to Pagano (1900). Further, intravenous injections of doses such as used in the vertebrals in several of the dogs were without effect on respiration. Nevertheless, it was deemed advisable here, as in the case of the carotid injections, to make injections after double vagotomy as well as double sinus denervation in several experiments, to exclude any possibility that effects of vertebral injections might be of vagus reflex origin.

Fig 5

Thirty-five injections were made in fifteen dogs. In this set of injections, as in that previous to vagotomy, several were made with one or more of the cephalic arteries occluded.

Seven injections were without effect in five dogs of this group. In each of two of the dogs, one ineffective injection was the only one made (2.0 cc. of 0.01 M. solution injected during 14 seconds into a 22 kgm. dog, and 1.0 cc. of 0.01 M. solution injected into a 13 kgm. dog, respectively). The cephalic arteries were all patent in both cases. In a third of these animals, body weight 24 kgm., with the sinuses tied off and procainized (hence carotid supply to the brain obstructed), 0.5 cc. and 1.0 cc. of 0.01 M. solution injected during 3 and 6.5 seconds, respectively, were both without effect. No other injections of this type were made in this dog. In the fourth, with all cephalic arteries patent, two 1.0 cc. injections of 0.01 M. solution (during 16 and 29 seconds, respectively) were without effect. In the same animal, however, four other injections of the same dose during 11 to 18 seconds caused definite effects. In the fifth and last dog in which there were any negative results, the dose was 0.4 cc. of 0.0025 M. solution injected during 5 seconds. The animal weighed 16.7 kgm., and both internal carotids were occluded. In the same animal five injections ranging in dosage from 0.1 cc. to 0.25 cc. of 0.01 M. solution each were definitely effective in stimulating the rate of respiration.

In only three dogs, then, were negative results only obtained, and in each of these but one or two injections were made. It is believed that larger doses, or the same or smaller doses with the opposite vertebral and carotid arteries all occluded, would have elicited effects. The ineffective doses here were of the same order of magnitude as those for injections made before vagotomy. As was the case for injections before vagotomy, the usual result of a vertebral injection was a definite effect on respiration. The effective dose ranged from 0.1 cc. of 0.01 M. solution injected during 2 seconds into a 16.7 kgm. dog with the internal carotid arteries occluded, to 2.0 cc. of 0.01 M. solution injected during 24 seconds into a 14.5 kgm. dog.

The latent period varied from 8 to 28 seconds, depending on factors mentioned in the previous section, with an average of 16 seconds taken from 23 measurable values. This average is seen to be of the same order as that for pre-vagotomy injections.

The effects themselves may in general be arranged into the same groups into which those of pre-vagotomy injections fell. Due, possibly, to the absence of the "selbsteuerung" action of the vagus nerves on respiration, the initial intense effect was frequently accompanied by or replaced by an increase in inspiratory tone, or by pauses in the inspiratory position (fig. 5, P, W). Some records showing effects falling into the various three groups are: group 1, fig. 5, P, Q; group 2, fig. 5, R, S, T; group 3, fig. 5, U, V, W.

This set of injections after vagotomy supplements those made with the vagus nerves intact, and demonstrates that the effects are not attributable to an inadvertent stimulation of the vagus system peripherally by cyanide.

C. Discussion. The invariable, intense, transient type of stimulation

of respiration resulting almost immediately after the injection of small doses of cyanide into the normally innervated carotid trunk, and eliminated by carotid denervation, is without doubt a reflex arising in this region. The constancy and importance of a carotid reflex mechanism of respiratory stimulation by cyanide, as reported by Heymans et al. (1931) and later by Owen and Gesell (1931) are unquestionable. The fact that the slightest trace of cyanide (0.0003 to 0.003 mgm. of the sodium salt per kilogram body weight of the animal), even as it suffers dilution in the carotid stream, is sufficient to excite this mechanism, is important.

It appears significant that, whereas doses 25 to 75 (average 33.5) times as large as the average minimal dose required for a carotid reflex effect, injected into a denervated carotid under conditions usually made most favorable for a diversion of the injection toward the Circle of Willis, were without effect in 71 per cent of the animals so used; in the case of vertebral injections, on the other hand, doses 2.5 to 75 (average 22.5) times as large as the average minimal reflex dose, in 84 per cent of animals, caused definite and sometimes excessive effects on respiration. Further, it is believed that doses within this range, if injected with more of the cephalic arteries occluded, would have been effective in those 4 dogs which were negative to the one or two vertebral injections made in each.

It appears, first, that Heymans was not altogether justified in using as a considerable portion of his evidence that the bulbar respiratory center is inexcitable by cyanide (or depressed by it), the fact that carotid injections after sinus denervation are usually (always, according to him) without a stimulating effect. The internal carotid vascular supply to the medulla appears to be too indirect and too variable in resistance, as well as subject to dilution by the spinal artery supply (and vertebral supply, when not obstructed), to allow for dependability in carotid injections for studying medulla responses.

Quite contrary to Heymans' statement that vertebral injections of cyanide are either without effect or depressing, results of 75 injections into 25 animals, either before or after vagotomy, or both, indicate only one pure depression, and that in an animal overanesthetized and failing. The effects of some or all the injections in 84 per cent of the dogs indicated stimulation in some or all phases.

There is, however, a decided difference between the carotid reflex effect and the vertebral effect. The former is invariably a sudden, intense, and transient simple stimulation, varying in intensity directly as the dose used without any great qualitative variation. The latter, on the other hand, varies qualitatively with a number of factors. First of all, it appears from the case of one dog, that an asphyxial, overanesthetized, or otherwise failing animal with its bulbar respiratory center suffering in common with other tissues, will respond by a simple respiratory depression. This is only to be

expected from the nature of the action of cyanide on cellular respiration (fig. 5, M). In some animals, probably those in which the medullary nerve cells have suffered least from the anaesthetic or from other experimental sources of anoxia, comparatively large doses may be injected into the vertebral artery with a correspondingly intense stimulation, not followed or accompanied by any phase of depression (fig. 5, P). Usually a small dose carefully injected results in a slight and gradual, but definite, increase in respiration, most commonly in rate (fig. 5, B, D, R, T), but frequently in amplitude (fig. 5, N). A larger dose in the same animal or the same or a smaller dose with more cephalic arteries occluded, ordinarily results in a more intense primary increase in rate, or in rate and amplitude, usually predominantly the former, followed or accompanied by a depression of amplitude which varies in degree with the dose used and the animal's susceptibility (fig. 5, A, C, E, F, H, I, J, K, L, O, Q, S, U, V, W). Unless the depression has been too pronounced (fig. 5, E, Q), and presumably the neurone cell bodies of the center damaged considerably, there is quite commonly a prolonged final swell especially in the amplitude of respiration (fig. 5, A, G, H, I, J, K, L, O, U, V, W).

The comparatively long latent period and the nature of the effects of vertebral injections point toward the probability that they are central, rather than the result of stimulation of some as yet unknown vascular reflex zone along the vertebral artery.

III. Femoral intravenous injections. A. *All innervation intact.* In the normally innervated dog, rapid intravenous injection of 2 to 3 cc. of 0.01 M. solution of sodium cyanide results in a sudden, intense, and transient stimulation of respiration of the type obtained on injection into the normally innervated carotid, and undoubtedly predominantly of sinus reflex origin (fig. 6, A, B, C). The latent periods of the effects observed from 20 injections in 18 dogs ranged from 8 to 16 seconds, with an average of 11.7 seconds. The rates of injection varied between 1.0 cc. in one second and 1.0 cc. in ten seconds.

B. *Carotid sinuses denervated.* After denervating both sinuses, 4 to 8 cc. of 0.01 M. NaCN solution invariably caused some sort of stimulation. The effects were not usually, however, of the simple type obtained with the carotid sinus innervation intact. The latent periods ranged from 8 to 25 seconds, with an average of 15.3 seconds (taken from 23 measurable values among 15 dogs). It is significant that somewhat over half these latent periods were within the range found for those where the carotid innervation was intact. The remainder were somewhat longer.

In the majority of effects whose latent periods were within the range found in the cases of intact carotid innervation, as well as in some whose latent periods were slightly beyond this range, two phases are more or less definitely discernible. In approximately three fourths of such two-phase

A.
2.0 cc. 0.01 M. NaCN Fem. V.
B.
3.0 cc. 0.01 M. NaCN Fem.
C.
2.0 cc. 0.01 M. NaCN Fem. V.
D.
6.0 cc. 0.01 M. NaCN Fem. Vein
(Sinuses Denervated)
E.
6.0 cc. 0.01 M. NaCN Femoral Vein
(Sinuses Denervated)
F.
6.0 cc. 0.01 M. NaCN Fem. Vein
(Sinuses Denervated)
G.
4.0 cc. 0.01 M. NaCN Fem. Vein
(Sinuses Denervated)
H.
5.0 cc. 0.01 M. NaCN Fem. Vein
(Sinuses Denervated)
I.
6.0 cc. 0.01 M. NaCN
Fem. Vein
(Sin. Den.)
J.
6.0 cc. 0.01 M. NaCN Fem. Vein
(Sinuses Den. — Vagi Cut)
K.
3.0 cc. 0.01 M. NaCN Fem. Vein
(Sin. Den. — Vagi Cut)
L.
3.0 cc. 0.01 M. NaCN Fem. V.
(Sin. Den. — Vagi Cut)
M.
4.0 cc. 0.01 M. NaCN Fem. Vein
(Sin. Den — Vagi Cut)
N.
2.0 cc. 0.01 M. NaCN Femoral Vein
(Sinuses Denervated — Vagi Cut)
O.
6.0 cc. 0.01 M. NaCN Fem. Vein
(Sinuses Den. — Vagi Cut)
P.
4.0 cc. 0.01 M. NaCN Fem. Vein
(Sinuses Denervated — Vagi Cut)
Q.
6.0 cc. 0.01 M. NaCN Fem. Vein
(Sin. Den. — Vagi Cut)
← One minute →

Fig. 6

results the primary phase was a decrease in rate, sometimes accompanied by an irregular increase in amplitude (fig. 6, D, E). In other cases where the effect was two-phased, the primary phase was a slight and gradual increase in rate and frequently also in amplitude (fig. 6, F, H). The second phase was a definite, sometimes quite marked and long-lived, stimulation of rate or of rate and amplitude. Where the primary phase was a gradual stimulation, it was marked off from the secondary by an abrupt acceleration of the stimulation, frequently initiated by a deeper breath. It appears justifiable to emphasize the fact that the total latent period until the *secondary* phase ranged between 20 and 44 seconds (except for one of 9 seconds in a small 9 kgm. dog), which will be seen to fall within the range of the latent periods of effects obtained with the carotid sinuses denervated and the vagus nerves cut.

It seems reasonable, therefore, that the primary effects in these double results were the beginning of vagus stimulations, probably at the cardio-aortic region in view of the fact that the latter, and not the pulmonary endings, have been found excitable as concerns respiration by 0.01 M. NaCN (Pagano, 1900) and further in view of the proximity of their latent periods to those for effects with the carotid innervation intact. It would seem that if the effects were pulmonary in origin, the average latency would be somewhat less, rather than more, than for the latter cases. As mentioned elsewhere, an inconstant effect of cyanide on respiration having its origin on vagus endings has been observed in this laboratory previous to this (Hertzman and Gesell, 1927).

Almost half the effects after double sinus denervation were simple increases in rate and amplitude, but more gradual and persistent than a carotid reflex effect (fig. 6, I). The latent periods for such effects ranged between 15 and 25 seconds, which, in view of the fact that many were below the range of those for effects after vagotomy, suggests that vagus effects were at least occasionally present among them, and that they merged indiscernibly into and were added to the secondary effect.

C. *Carotid sinuses denervated and both vagus trunks rendered non-functional.* As mentioned above, the total latent period of the second phase of effects obtained from femoral intravenous injections after double sinus denervation fell within the range of latent periods for effects obtained after double vagotomy in addition. That range was 20 to 44 seconds, with an average of 31 seconds (taken from 37 measurable values among 12 dogs). The obvious suggestion is that the second phase of results before vagotomy is the overlapping of a continuing vagus effect onto a later effect able to be elicited *after* vagotomy. The nature of this later effect, now to be considered, and its late occurrence, are evidence that it is not an additional specific vascular respiratory reflex. Although these experiments themselves do not eliminate the possibility that the effects may originate from

generalized sensory endings, particularly in vessel walls, it is ordinarily believed that cyanide solutions of the strength used do not affect respiration over such general afferent paths. The statement has been made elsewhere that in a systematic examination of the effects of strong solutions of cyanide injected locally at various points in the vascular system, Pagano (1900) was able to conclude that the cardio-aortic region and cephalic arteries are the *only* vascular zones where cyanide may reflexly affect respiration. It seems justifiable on these bases to consider any respiratory effects obtained upon the intravenous injection of 0.01 M. cyanide after both double sinus denervation and double vagotomy, as either central effects, or results of enhancing afferent and efferent impulses of a center.

Heymans et al. (1931) state that such intravenous injections after denervation are either without effect on respiration, or depress it. As was the case for the vertebral injections, and for a small number of the denervated carotid injections, the results now to be given are in direct contradiction with those of Heymans.

Stimulations, in some or all phases of the effects, were obtained in every one of the fourteen dogs so used, with doses ranging from 1.5 to 10.0 cc. of 0.01 M. solution. From one to six injections were made in each animal, with as many consecutive positive effects.

As was the case for the vertebral injections, the results of these intravenous injections varied, though always containing a stimulation in some phase.

Frequently the effect was a simple increase in rate and amplitude, more smoothly appearing and disappearing than the carotid reflex effect (fig. 6, J, K). Another type of effect obtained was an increase in rate and amplitude accompanied by considerable increase in general inspiratory tonus (fig. 6, M). A third type of result was an increase in rate alone (fig. 6, L). As was seen to be the case for vertebral injections, large doses frequently caused an increase in rate accompanied by a depressed amplitude (fig. 6, P).

In several cases especially with large doses, the results were complicated by pauses in the inspiratory position of varying durations, as was also frequently observed in vertebral effects after vagotomy. It is believed that these pauses may signify stimulation, and might have been replaced by an increase in rate with the "selbsteuerung" vagus action intact (fig. 6, N, O). Another type of effect observed included both the increase in general tonus and the inspiratory pauses (fig. 6, Q). It may be seen from the figures that as soon as the raised line of expiration (increased tonus of inspiratory muscles) subsides, there is a persistent, definite stimulation. It appears that the region on such a record where the tonus increase appears merely signifies a modification of the beginning of a prolonged stimulation.

In connection with these tonus increases observed with large intravenous injections after double sinus denervation and vagotomy, it is of interest

to recall the work of Gay (1930), who observed that the intravenous injection of cyanide into dogs increases the effectiveness of submaximal stimulation of myo-neural mechanisms, particularly where a reflex arc is involved (synapses). In the light of this observation the probability may be considered that the increased tonus resulting in less complete expirations may be the result of enhancing the effectiveness of the waning inspiratory motor impulses at the end of expiration. It appears equally possible that the waxing impulses during inspiration may be enhanced in effectiveness, and to an extent greater than the waning ones at the end of expiration in the same proportion as the original impulses compare with each other at the end of inspiration and expiration, thus accounting for at least some of the increase in amplitude constituting the stimulation. It is, however, difficult to account for the increases in rate observed, in this manner, unless it be that cyanide may have a similar enhancing influence upon the effectiveness of proprioceptive impulses from the muscles of inspiration. In themselves, these experiments do not allow a statement as to how much of the effects may be due to a central action of the cyanide, at a "respiratory pace-setter" (Gesell and Nyboer, 1929), and how much to an increase in the effectiveness of neural impulses to and from such a center. This much is clear: Judging from the vertebral effects already discussed and from the 4th ventricle effects to be considered later, some part of the effects of intravenous injections must be central.

D. *A comparison of sensitivity to cyanide before and after sinus and vagus nerves are removed.* In five dogs an attempt was made to determine the minimal dose of cyanide injected intravenously required to just definitely stimulate respiration, first before and then after severing the sinus and vagus nerves, or the equivalent. The results are shown in table 1. It is seen that somewhat less than three times as large a dose, on the average, was required after denervation as before. Obviously these results do not afford a direct comparison of the sensitivity of the center, and of the reflex zones together, to cyanide, since the extent to which the center is responsible for the effects after denervation is not determined. At best, one may say that these five experiments indicate that the animals' threshold to respiratory stimulation by cyanide is approximately trebled after double sinus denervation and double vagotomy.

IV. Injections of cyanide into the fourth ventricle. Even though the nature and the latent periods of the results obtained after injections of cyanide into a vertebral artery would preclude the probability that they are reflex effects, the possibility of a vascular reflex zone along the vertebral course is not definitely excluded.

With such a possibility in mind fourteen experiments have been done in which the injections were made directly onto the floor of the fourth ventricle.

With the animal's head held in a suitably flexed position, an approach was made through the muscles in the dorso-medial occipito-atlantal region by means of thermo-cautery. A free opening was made through the occipito-atlantal ligament and dura mater, through which a small, smooth-ended, double glass tube was inserted. The tube was carefully worked between the cerebellum and the dorsum of the medulla, through the tela chorioidea and into the ventricle. One passage of the double tube was connected with a burette from which the cyanide solution could be injected, and the other to a burette containing saline solution. The possibility that physical and extraneous chemical stimuli might enter as a factor into the effects from cyanide injections could thus be frequently ascertained by the injection of similar or larger quantities of saline of the same temperature

TABLE 1

DATE	WEIGHT	INNERVATION INTACT			SINUS AND VAGUS NERVES NON-FUNCTIONAL		
		Duration of injection	Volume and normality of injection	Milligrams per kilogram of body weight	Duration of injection	Volume and normality of injection	Milligrams per kilogram of body weight
	kgm.	*seconds*	*cc. N.*		*seconds*	*cc. N.*	
1/ 9/32	24.5		1.0 –0.01	0.020	9.0	2.0–0.01	0.040
1/14/32	21.2	5.0	1.0 –0.01	0.024	9.0	3.0–0.01	0.070
					12.0	4.1–0.01	0.093
1/23/32	21.5	2.5	1.0 –0.01	0.023	3.5	3.0–0.01	0.069
1/28/32	15.0	1.0	0.85–0.01	0.028	3.0	3.0–0.01	0.100
2/ 1/32	16.7	1.5	0.7 –0.01	0.021	1.5	1.0–0.01	0.029
Mean, mgm./kgm.....				0.023			0.064

(room temperature). Moreover, large cyanide injections could be quickly diluted and partially washed out with the saline.

From one to twenty-three injections of cyanide solution in physiological saline were made into the fourth ventricle of each of 14 dogs so prepared.

Effective doses ranged from 0.75 cc. of 0.0008 M. solution during 3 seconds to 2.0 cc. of 0.01 M. solution during 21 seconds. Such large doses as the latter resulted in a final depression of the respiration to apnea. The latent periods of the effects were extremely variable as might be expected, since due to anatomical variations, the stream of injection was not directed toward any one region in the rhomboid fossa constantly throughout the series of experiments, and since probably no constant stratum of fluid and tissue was present through which the chemical diffused before reaching an excitable region.

As in the case of vertebral effects, the results obtained in these experi-

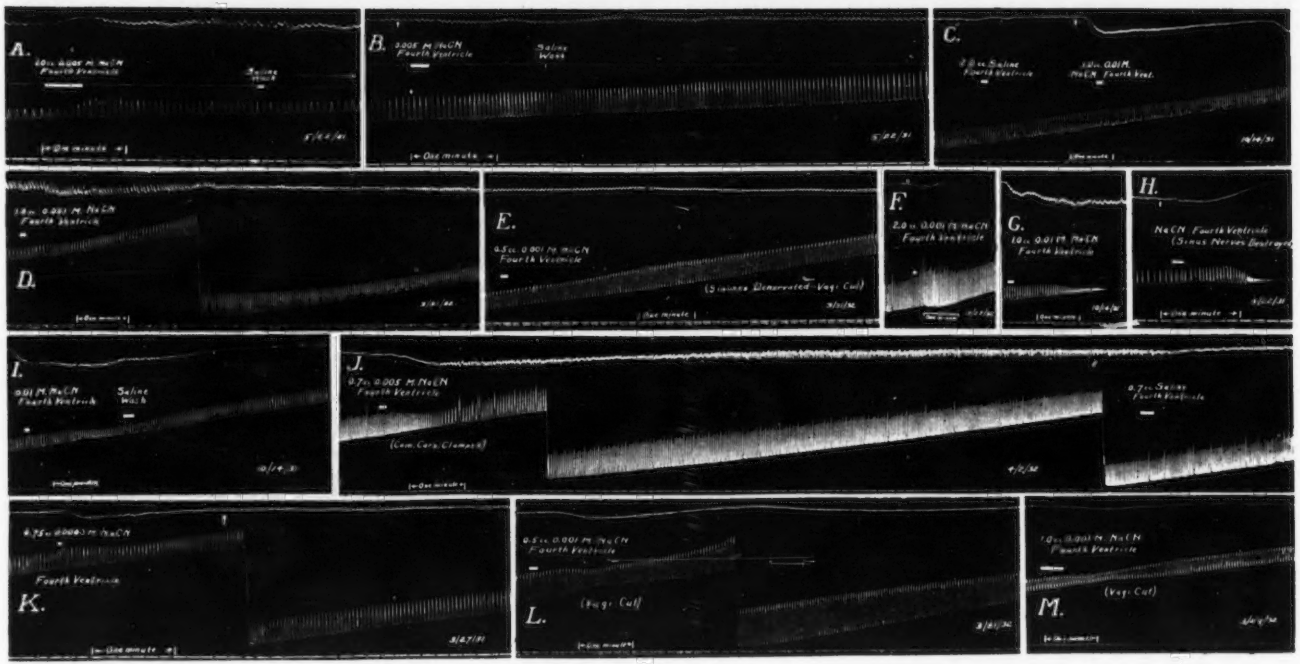

Fig. 7

ments were variable. Because far more injections were usually made per animal than in the case of the vertebral artery injections, it is even more obvious in the fourth ventricle injections that the strength, quantity, and rate of injection of the dose used were largely responsible for the variations. As was also observed in the case of the vertebral injections, the most common result was an increase in respiratory rate in some or all phases of an effect usually not otherwise simple.

When the dose used was small, or injected slowly, or subsequently to flushing out the ventricle or filling it with saline, the effect was frequently a simple stimulation of respiration. In such cases the stimulation was usually either an increase in rate alone (fig. 7, B, C) or more frequently an increase in both rate and amplitude (fig. 7, A, D, E, F). In one of the animals above (fig. 7, F) ten consecutive injections of 1.0 to 2.0 cc. of 0.001 M. solution each resulted in an increase in both rate and amplitude. The effects of doses that were of greater strength, injected more rapidly, or otherwise able to cause a more intense effect, were usually characterized by a decreased amplitude following the initial stimulation in rate or in rate and amplitude, or accompanying an increase in rate. In such cases the increased rate usually became more marked during the amplitude depression. Following this secondary amplitude depression, there was either a return of both rate and amplitude to the normal, or, as was more commonly the case, a more or less marked and prolonged secondary increase in both amplitude and rate, or in one or the other alone, or occasionally an increase in amplitude at a slower rate than normal. Such results are shown in figure 7, I, K, L, M. When doses causing still more intense effects were used, the secondary amplitude depression went more or less abruptly into an apneic phase (fig. 7, G, H). In a very few cases where the dose was large enough to cause a secondary depression in the amplitude there was either no accompanying increase in rate, or a decrease (fig. 7, J).

Occasional pure depressions were observed. In one animal such was the case a few minutes after sodium sulfide had been injected. In one dog a case of depressed rate, and in another, three of depressed amplitude, were observed when the common carotid arteries were occluded. Although in these two animals such *pure* depressions were observed only under these conditions of carotid occlusions, in other cases under the same conditions, in these as well as other animals, stimulations were observed. In one dog, the first and smallest dose used caused a simple decrease in both rate and amplitude, whereas all subsequent doses resulted in stimulations in some or all phases of the effects. In two animals such pure depressions were observed after both sinus and vagus nerves were severed, whereas in other individuals, these denervations appeared to have no consistent qualitative effects on the results obtained.

Considering cases where the total course of the effect of the injection was

allowed to run on without experimental interference, and excepting those effects the doses for which were so large as to result in respiratory failure, for 74 per cent of the injections, the total effect from the time of the injection to a return of respiration to a constant rate and amplitude (commonly several minutes) was an increase in the volume exchange of respiration, frequently very great. In 17 per cent the total effect was a decrease. In 9 per cent the total effect was questionable. In two of the animals total increases only resulted (6/1/31, 3/22/31). In three animals, in each of which but one injection was made and in two of which that injection was so large as to result in respiratory failure, the only total effect observed was decrease in volume exchange. In the remaining dogs, where both total increases and total decreases resulted from the injections, the decreases usually were more numerous toward the end of the rather vigorous experiment, when the animal was presumably not in as normal a condition as at the beginning. This observation is in accord with that in connection with the vertebral injections where a simple depression was observed only in one experimentally or anesthetically asphyxial animal.

SUMMARY AND CONCLUSIONS

The carotid reflex mechanism of respiratory stimulation by cyanide salts injected vascularly, reported by Heymans et al., was invariably and readily demonstrable. When injected directly into the common carotid artery of dogs, traces of sodium cyanide as small as 0.0003 mgm. to 0.003 mgm. (averaging 0.001 mgm.) per kgm. body weight were sufficient to excite respiration over this reflex path. These carotid reflex effects were abrupt, intense, and transient.

Cyanide was capable of affecting respiration reflexly in a less intense and more variable manner over the vagus nerves. The response attributed to vagus stimulation by the salt was either a slight stimulation of rate and amplitude or of one or the other alone, or a depression of rate without change in amplitude or with irregularly increased amplitude. Reasons are given for believing that this vagus effect is of cardio-aortic origin.

After double sinus denervation, with or without double vagotomy, common carotid injection of doses of cyanide 25 to 75 times as large as the average minimal dose required before denervation only occasionally affected respiration even though in the majority of cases the external carotid on the side of injection was occluded and in some cases also the vertebral and opposite common carotid arteries. The occasional stimulations observed were sometimes marked. This inconstancy may be due to the fact that the carotid system supplies predominantly the superior regions of the brain, and that the posterior communicating artery of the Circle of Willis is of rather small and variable calibre. The difficulty in shunting blood cau-

dally through it by occlusion of vertebral arteries indicates the rôle that the spinal arteries may play under such conditions, in supplying the medulla.

Doses of cyanide 2.5 to 75 (averaging 22.5) times as large as the minimal effective carotid reflex dose ordinarily resulted in stimulations of respiration in some or all phases when injected into the vertebral artery. The stimulations were increases in rate or amplitude or both, most consistently in rate. The stimulation was followed either by a return to normal, or by a depression. The depression, in intense dosage, reached apnea. Following such a secondary depression there was frequently a late, prolonged, more or less marked increase in amplitude, more than could be attributed to the physiological effects of the previous depression. Variations in types of effects were largely due to variations in the dose used, with reference to the animal's sensitivity.

Injections of cyanide directly into the fourth ventricle produced effects on respiration qualitatively resembling those from vertebral artery injections. Such direct central injections were frequently effective with doses of the order required for a carotid reflex effect.

After both sinuses were denervated and the vagus trunks severed, or the equivalent, effective intravenous (femoral) injections of cyanide stimulated respiration in some phase or all. Changes in inspiratory tone and prolonged inspirations are briefly discussed.

Although the occasional stimulations from carotid injections after denervation, and the more consistent stimulations from vertebral artery injections and flooding of the fourth ventricle indicate a stimulating action of cyanide at intracranial centers concerned with respiration, what portion of the effect is attributable to direct action on a respiratory "pace-setter," or to an enhancing action on afferent impulses to such a primary region is not determined.

In five dogs the threshold to respiratory stimulation by cyanide was approximately trebled by double sinus denervation and double vagotomy.

REFERENCES

Danielopolu, D., I. Marcou and E. Manescu. 1930. Zeitschr. f. d. ges. exp. Med., lxx, 268.

Danielopolu, D., I. Marcou and G. G. Proca. 1931. Compt. rend. Soc. de Biol., cvi, 734.

1932. Zeitschr. f. d. ges. exp. Med., lxxxi, 273.

Danielopolu, D. and I. Marcou. 1932. Compt. rend. Soc. de Biol., cix, 761.

Dautrebande, L. 1931. La Méd., September 1931, p. 694.

Gay, H. 1930. This Journal, xcv, 519.

Gesell, R. 1928. This Journal, lxxxvi, 164.

Gesell, R. and J. Nyboer. 1929. Bull. Battle Creek San. and Hosp. Clin., xxiv, 394.

Hering, H. E. 1927. Die Karotissinusreflexe auf Herz und Gefässe. Dresden und Leipzig.

HERTZMAN, A. B. AND R. GESELL. 1927. This Journal, lxxxii, 608.
HEYMANS, C., J. J. BOUCKAERT AND L. DAUTREBANDE. 1931. Arch. internat. de Pharm. et de Thérap., xl, 54.
HEYMANS, J. F. AND C. HEYMANS. 1927. Arch. internat. de Pharm. et de Thérap., xxxiii, 273.
OWEN, H. AND R. GESELL. 1931. Proc. Soc. Exp. Biol. and Med., xxviii, 765.
PAGANO, G. 1900. Arch. ital. de Biol., xxxiii, 1.
WINDER, C. V., H. OWEN AND R. GESELL. 1932. This Journal, ci, 103.

THE SEAT OF ACTION OF SULFIDE ON PULMONARY VENTILATION

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Received for publication May 19, 1933

It is well known that sulfides injected into the blood stream or hydrogen sulfide administered by way of the lungs has an intense stimulating action on pulmonary ventilation. Since Haggard (1921) has shown that Na_2S is immediately and almost completely hydrolyzed on entering the blood stream, releasing an equivalent of H_2S, effects of Na_2S are believed to be due to the H_2S which it releases. Haggard believes that since H_2S does not enter into combination with hemoglobin except under abnormal conditions of special etiology, the physiological effects of Na_2S or H_2S are due to H_2S in solution in body fluids.

Haggard and Henderson (1922), from observing the effects of sulfide in dogs before and after vagotomy, conclude that the seat of stimulation of respiratory movements by the chemical is at vagal endings, probably pulmonary. However, Nack and Wulp (1929), working in this laboratory, were unable to obtain any evidence of a vagal pathway for the respiratory effect of Na_2S (H_2S). On the contrary, their results established the importance of a cephalic seat of action which they, not knowing at that time of carotid sinus (and glomus caroticum) respiratory reflexes, termed "central."

Soon after Heymans, Bouckaert and Dautrebande (1930) emphasized the importance of carotid reflexes in physiological alterations of pulmonary ventilation, these investigators (1931) and somewhat later Owen and Gesell (1931) reported an intense reflex stimulating action of Na_2S on pulmonary ventilation, arising at the carotid zone. Heymans' group, in their final report, and Owen and Gesell in reporting preliminary experiments, agree that carotid injections of Na_2S under such conditions that the carotid innervation is not stimulated result in little or no augmentation, or in depression of respiratory movements. Both report similar results for intravenous injections of Na_2S after cutting the sinus and vagus nerves.

However, in a few preliminary experiments wherein Na_2S was injected directly onto the floor of the fourth ventricle, the procedure for which is briefly described in a preceding paper (Winder, Winder and Gesell, 1933),

Owen and Gesell found consistent, immediate, well sustained and marked augmentation of ventilation.

In view of these apparently definite effects which would indicate a central action of the chemical, and further in view of the fact that effects of vertebral arterial injections were not studied in the preliminary experiments here, the work in this laboratory has been continued in an attempt to correlate the apparent discrepancies concerning an intracranial action of sulfide.

METHODS. Dogs of various varieties and sizes were used (11.5 to 32.0 kgm.). The usual morphine and urethane anesthetic employed in this laboratory was used. Blood pressure was recorded by means of a mercury manometer connected with a femoral artery, and respiration by means of the spirometer of a rebreathing system of the type standard to this laboratory. A tracheal cannula in which dead-space was reduced to a minimum served as a connection with the rebreathing circuit.

Vascular injections of Na_2S in physiological saline or Locke's solution were made into the common carotid artery, vertebral artery, and femoral vein, before and after sinus denervation and vagotomy. Hering's (1927) method for carotid denervation was employed (in this procedure the occipital artery was obstructed). Fourth ventricle injections were made as described in the preceding paper (1933).

In the figures, *S* within a circle indicates double sinus denervation, and *X* within a circle, double vagotomy.

VASCULAR INJECTIONS. A small quantity of Na_2S injected rapidly into a common carotid with normally innervated carotid region invariably results in an almost immediate, abrupt, intense, and transient augmentation of ventilation. Both rate and depth are affected (fig. 1, A, B, C, D, E). Frequently, this intense hyperventilation is followed by a compensatory falling off in ventilation (fig. 1, A, B, E), presumably either of acapnic origin, or a result of augmented vagus inhibition (Hering-Breuer) from the greater inspirations, or both.

The time interval between the beginning of the injection and the first effect was found to vary between 2 and 5 seconds, depending largely on the rate of injection and strength of dose used. The average latent interval was 3.25 seconds.

The effects after vagotomy are similar (fig. 1, F, G, H) except that inspiratory tetanus may characterize the beginning of an intense effect (fig. 1, F, and H), and the recession may be less abrupt (fig. 1, F).

In each of seven experiments a series of decreasing doses was injected in an attempt to determine the minimal effective dose. The minimal effect was usually a single considerably higher inspiration consistently elicitable, or a slight definite swell in amplitude over more than one inspiration. The minimal dose varied from 0.075 cc. to 0.24 cc. of 0.001 M. solution. The

average was 0.14 cc. of 0.001 M. solution, or 0.01 mgm. of Na_2S. In milligrams per kilogram body weight, the range was 0.00025 mgm. to 0.00134 mgm. per kilogram, with an average of 0.00059 mgm. per kilogram. These quantities are but slight traces of the chemical. Vagotomy did not appear to alter the *order* of magnitude of minimal dose. It might have lowered it slightly.

It seems of interest to recall that for similar injections of NaCN into the patent common carotid with its sinus region normally innervated, the effects were quite similar to these for Na_2S (Winder, Winder and Gesell, 1933). The latent interval range for cyanide was 2 to 6 seconds as compared with 2 to 5 seconds for sulfide. The average minimal effective dose

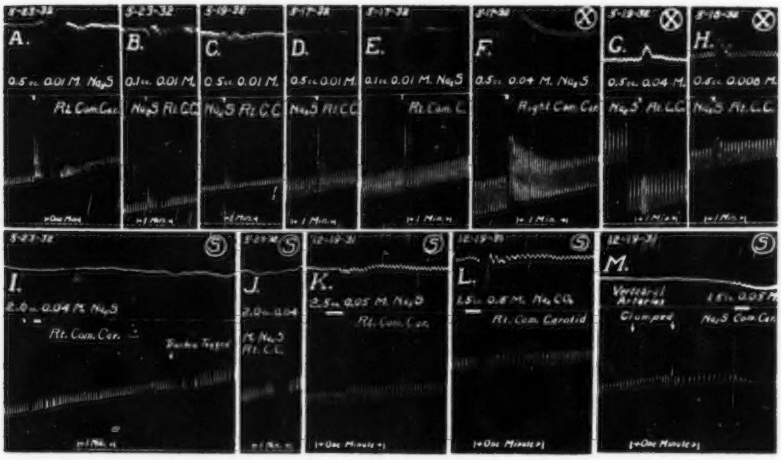

Fig. 1

found for cyanide was 0.001 mgm. per kilogram body weight, as compared with 0.0006 mgm. for sulfide. In short, the complete similarity of cyanide to sulfide in its action on pulmonary ventilation at the carotid zone seems quite apparent. It is also obvious enough that this reflex mechanism is important for both. That this intense carotid effect certainly is a reflex from the carotid sinus region is easily demonstrated by its elimination on denervation of that region.

This work is particularly concerned, however, with the possibility of a central stimulating action of sulfide. Carotid injections after sinus denervation of the type employed have been found in the preceding series of experiments with cyanide to be unreliable in directing the injected chemical toward the medulla, so that little will be said about such injections of

Na_2S. As mentioned above, Heymans and his collaborators, and Owen and Gesell here, agree that little or no stimulation, or depression of respiration results from such injections.

In eight experiments of the series now being considered this type of injection was used 28 times with doses of 1.0 to 2.5 cc. of 0.04 M. solution, excepting one 5.0 cc. and one 6.0 cc. dose. No effect whatever was observed in four of the eight experiments. In two other experiments, for no constant apparent reason, one injection would be followed by a slight indefinite swell, so insignificant as probably not to be appreciable from a reproduction of the record, whereas the next would have no effect whatever. One injection appeared to cause a very slight depression and smoothing of the height of inspirations.

In the seventh of the eight experiments ordinary doses caused either irregularity in rate and depth of breathing so as not to affect greatly one way or the other the total gaseous volume exchange, or abrupt apnea (fig. 1, I, J). It was observed that (general?) peripheral stimulation, as tugging at the trachea (fig. 1, I), femoral intravenous injections after sinus denervation and vagotomy (fig. 3, P), and vertebral intra-arterial injections (fig. 2, G, H) caused similar types of phenomena in this animal. The breathing appeared very prone to complete cessations.

In the last of the eight animals, with the external carotids occluded and the denervated sinus regions bathed in cocaine solution, 2.5 cc. of 0.05 M. Na_2S solution injected during 15 seconds on the right side resulted in a definite slowing of rate and increase of amplitude 10 seconds after the beginning of the injection (fig. 1, K). It is noteworthy that a similar though more marked effect was elicited by injection of Na_2CO_3 (base) into the same artery under the same conditions (fig. 1, L). Subsequent injections of 2.0 cc. each on either side produced no definite effect. Toward the end of the experiment 1.5 cc. was injected while both vertebral arteries were occluded (fig. 1, M). As may be seen from the figure, occlusion of the first vertebral artery resulted in a definite stimulation of respiration. As the marked curtailment of blood supply was increased by occlusion of the second vertebral, however, respiration began to fail, so that by the time the injection was made, the added depressive effect of the sulfide resulted in complete failure.

For some of these injections the vagus trunks were intact, for others severed. No difference could be observed attributable to the vagi. Occlusion of the external carotids had no consistent influence on the results in those cases where such a procedure was used.

It is to be seen, then, that no addition can be made to Heymans' statement or to the preliminary statement from this laboratory, that carotid injections after double sinus denervation are either without effect on respiration, or cause insignificant stimulations, or depressions. The occa-

sional effects obtained appear to be of intracranial origin, though whether they are indications of stimulation of superior regions of the brain, or of a descent of the injected dose down through the small posterior communicating artery of the Circle of Willis to the region of the respiratory nuclei in the brain stem, cannot be said. In any event, it is to be borne in mind that the carotid injections apparently would not readily have reached the region of the medulla of dogs (Winder, Winder and Gesell, 1933). Of course, there is also the possibility that some weakly differentiated reflexes arising from vessel walls might have originated some of the insignificantly small effects. Finally, it is to be emphasized that at least after entering the blood stream Na_2S hydrolyzes almost completely with the release of an equivalent of the strong base, NaOH. Hence, the basicity of the larger doses may account for some of the positive effects observed, particularly the slowing with increased amplitude considered in connection with the eighth animal (fig. 1, K).

Of any of the types of vascular injections able to be conveniently performed, vertebral intra-arterial injections are probably of most value in studying the effect of a chemical in question at the inferior part of the brain. From one to sixteen such injections were made in each of nine experiments. They were made in the animal with normal innervation, after vagotomy, after sinus denervation, and after both vagotomy and sinus denervation. During the injections the other cephalic arteries were all patent. The rate of injection was 4.5 to 35 seconds per cubic centimeter, averaging 8.2 seconds.

In order that some idea may be had of the size dose needed for an effect, the range of ineffective and effective doses in some of the animals will be considered. A dose as large as 0.2 cc. of 0.04 M. solution in one normally innervated animal (23.5 kgm.) was without effect. Doses of 0.5 cc. before and after severing the vagus trunks and after denervating the sinuses produced definite effects in this dog. In another case (dog, 16.5 kgm., normally innervated), 0.4 cc. of 0.008 M. solution was without effect. Subsequently, doses of 0.5 cc. of the same solution, before and after cutting the vagus trunks and after denervating the sinuses, caused effects on respiration. In a third dog (19.0 kgm.) 0.5 cc. of 0.008 M. solution caused a just appreciable effect with the innervation intact. After cutting the vagus trunks, when respiratory movements were large and irregular, 0.5 cc. of 0.04 M. solution was required for an effect that was discernible. In a fourth case (25.0 kgm.), 0.45 cc. of 0.008 M. solution was just definitely effective both before and after cutting the sinus nerves. After severing the vagus trunks in addition, this dose caused no obvious effect; 0.85 cc. did have an effect. In another of the animals (26 kgm.), while the innervation was normal 0.3 cc. of 0.01 M. solution elicited a minimal effect, whereas after denervating the sinuses, and also after cutting the vagus trunks in

addition, 0.1 cc. was definitely effective. In a sixth dog (14 kgm.) 0.3 cc. of 0.01 M. solution was just definitely effective with innervation all intact, after denervating the sinuses, and after severing the vagus trunks in addition.

In short, the usual minimal dose of sodium sulfide rapidly injected into the vertebral artery required for an effect on pulmonary ventilation approximated the equivalent of 0.5 cc. of 0.01 M. solution.

The time interval between the beginning of the injection and the first appearance of the effect ranged from 6 to 16 seconds, depending on the rate of injection, strength and quantity of dose, etc. The average was 10.1 seconds.

The absence in the effects of any sign of the immediate, intense and abrupt increase in amplitude characteristic of the carotid reflex effect shows, in itself, that there was no recurrent arterial circulation of the injections to the carotid regions under the conditions employed (viz., care not to partially occlude the common carotid on making the injections), so that the results of injections made with the carotid innervation intact are as significant as those obtained after denervation.

Sulfide effects in the vertebral artery were more easily predictable qualitatively on the basis of the dose used than was the case for cyanide (Winder, Winder and Gesell, 1933). The almost consistent domination by stimulation found to be characteristic of the variable effects of vertebral injections of cyanide was lacking in the case of sulfide. In figure 2 are represented the types of effects observed.

Figure 2, A, C, D, F, I, L, N, and P show the effects of minimal doses. One type of minimal effect was a very slight increase in general tone of inspiratory muscles, accompanied by a more or less evident slight increase in rate (A, C, D). This effect was observed in the normally innervated animal, and after cutting the vagus nerves in the same animal (A, C). In another dog in which this sort of effect was obtained with the innervation intact, after destroying the sinus innervation a very much smaller injection caused a striking increase in both rate and amplitude, besides a more prolonged increase in general tone (D, F). In another normally innervated animal, an effect even more slight than those shown in A, C, D, or F, was a very small increase in rate without the tonus increase (I). After destroying the sinus innervation and cutting the vagus trunks of this dog, a somewhat larger dose caused a slight smooth swell in amplitude, in addition to a measurable increase in rate (L). Another type of minimal effect, observed in one dog both while normally innervated and after denervation of the sinuses, was a transient depression in amplitude accompanied by no change, or by the slightest increase, in rate (N, P). The effect appears a little more definite after the denervation (P).

More pronounced effects are shown in figure 2, J, K, M, O, R, S, T, U.

One may see that the one obvious common characteristic of these moderate effects is the notch-like depression of the record of amplitude, or depth of breathing, accompanied by a decreased rate. Preceding this depression there was quite commonly the more or less evident increase in tonus or rate, or both, that was seen to have constituted several of the minimal effects. Sinus denervation or vagotomy appeared to have no consistent qualitative influence on the effects, as one may see from the three series of

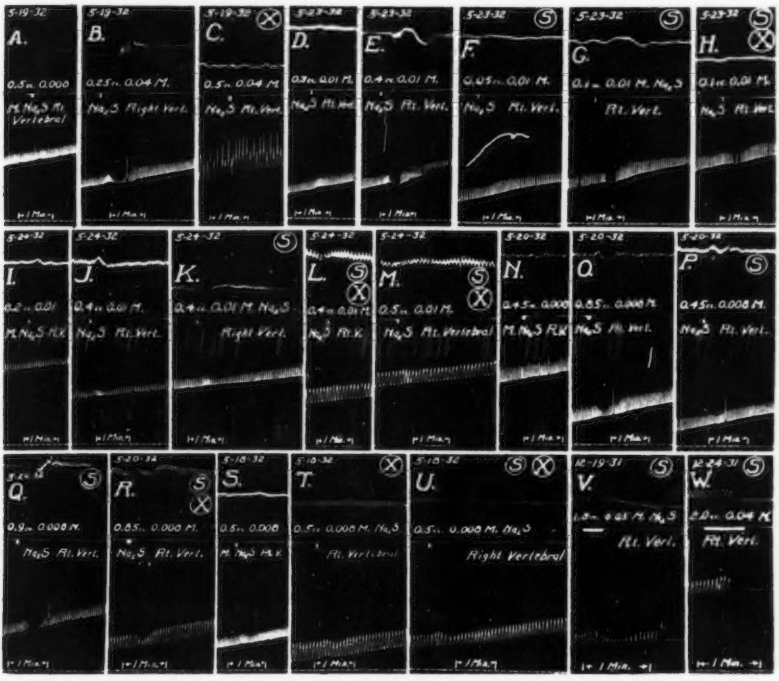

Fig. 2

figures: J, K, and M from one animal; O and R from another; and S, T, and U from still another.

Large injections (with reference to the particular animal's sensitivity) accentuated the various phases of a moderate effect. Figure 2, B, E, G, H, Q, V, W illustrate this. It is particularly obvious that the depression phase is more marked. A sufficiently large dose may result not only in a short apnea at this stage (E, G, H, Q), but even in sustained respiratory failure (V, W).

In several cases an overshooting recovery from the depression phase was observed, especially if the latter was marked or led to apnea (B, E, G, H, O, Q, R). A close examination of the records reveals, however, that the overshooting, when present, together with the slight primary stimulation, if present, is seldom if ever sufficient to compensate for the depression; consequently the total effect upon ventilation from the beginning of the injection until a return of respiration to a constant rate and depth was a reduction, except in those cases where the effect was just minimal.

One might summarize the respiratory effect of sulfide injected into the vertebral artery as follows: The minimal effect may be a slight increase in rate, or in rate and amplitude, commonly accompanied by more or less increase in general tonus of inspiratory muscles so as to result in a slightly larger mean pulmonary volume; or it may be a slight transient reduction in depth of inspiration, with or without a very slight acceleration in rate. A moderate effect appears always to possess a phase of decreased depth of inspiration associated with more or less slowing of the rate. Preceding the depression there is commonly a phase of slight increase in rate, or rate and amplitude, frequently associated with an increase in mean pulmonary volume. Following the depression there may be a simple return to normal, or an overshooting in rate or amplitude or both. Such overshooting, if present, together with the slight and short-lived primary stimulation, when present, is not sufficient to compensate for the depression, so that the total net effect is one of reduction of pulmonary ventilation. Presumably the overshooting represents a partial physiological adjustment to the effects of the previous reduced ventilation. More intense effects are similar, except that the various phases are accentuated, particularly the depression phase, which may lead to apnea. With extremely large doses (1.5 to 2.0 cc. of 0.04 M. solution) the apnea may be the beginning of respiratory failure.

It is supposed that this very slight respiratory stimulation with minimal doses, or depression or sustained injury to respiration with moderately or excessively effective doses, is the expression of an action directly at brain-stem nuclei concerned with respiratory movements. This seems necessarily the case for sustained injurious effects. If the slight and inconstant stimulation phase represents an additional vascular reflex, it is quite unlike the intense carotid reflex (general vessel sensory endings?). In view of the fact that sulfide (like cyanide and ordinary low oxygen administration) appears from the work of Gay (1930) to enhance the effectiveness of neural impulses passing synapses, if the action is to be attributed wholly to an influence on afferent impulses to the respiratory nuclei there would need be a selectively stronger action on inhibitory impulses, which seems unlikely. Neither of the latter two explanations is adequate alone. Either or both may possibly operate along with a direct effect at the nuclei.

Although carotid injections with the sinus innervation intact, and vertebral injections, afford more or less direct means of studying the action of the chemical injected at the carotid reflex zone and presumably in the brain stem, respectively, there is in these procedures no common basis upon which we may study relative sensitiveness, or importance of one or the other mechanism as concerned with the presence of the chemical generally in the blood stream. For this reason many femoral intravenous

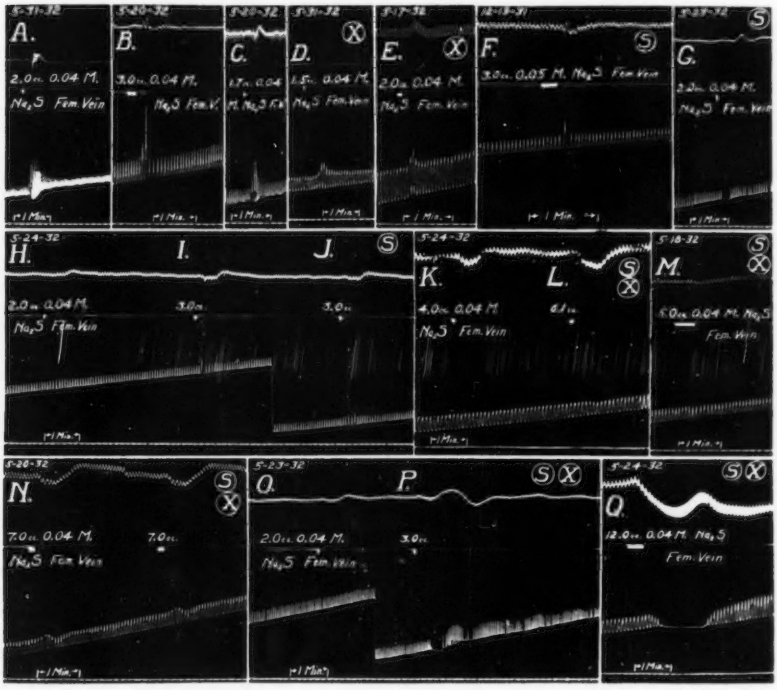

Fig. 3

injections were made, first in the normally innervated animal, then after sinus denervation or vagotomy, and finally after both sinus denervation and vagotomy. This procedure has also yielded some interesting information concerning vagus effects of sulfide.

Doses of 2 to 3 cc. of 0.04 M. Na_2S injected rapidly (1.0 cc. in 1 to 5 seconds) into the femoral vein of the normally innervated dog result in abrupt, marked and transient augmentations of ventilation. As is the case with carotid injections, both rate and amplitude are usually affected,

but especially the latter (fig. 3, A, B, C). The results closely resemble those of injections into a normal carotid system. As was observed for the latter, post-excitation falling off in respiratory movements was frequently present (fig. 3, B). No considerable difference in intensity of the effect or in its latent interval was observed after vagotomy, except an absence of post-excitation depression (fig. 3, D, E). This is in accord with Nack and Wulp's work.

Whether the absence after vagotomy of the post-excitation quiescence in movements was due entirely to the fact that effects as intense as those before vagotomy were not elicited, hence acapnia not so pronounced, or

TABLE 1

DATE	NORMAL INNERVATION		SINUSES DENERVATED	
	Mean arterial pressure before injection	Latent interval	Mean arterial pressure before injection	Latent interval
	mm. Hg	*seconds*	*mm.* Hg	*seconds*
5/20/32	124	15.0	139	10.0
	123	15.0	126	11.5
	108	13.0	132	13.0
5/23/32	106	12.0	100	11.0
			106	11.0
			110	11.0
			110	12.0
			110	11.0
5/24/32	106	14.0	74	15.0
			71	14.0
			70	14.0
			61	14.0
Mean.....	113	13.8	103	12.3

partially to the absence of vagus "after-effect" associated with hyperactive inspirations, could only be hazarded.

When, however, the innervation of the carotid sinus region was destroyed and the vagus trunks left intact, doses of the order indicated above produced an altogether different type of effect (fig. 3, F, G, H, I, J). It consisted in most cases of a definite slowing in rate accompanied by more or less increase in amplitude. Especially with larger doses, isolated deeper breaths appeared in the course of the effect. In the case of one dog (fig. 3, G), which was found in considerations mentioned above to be very prone to complete cessations of breathing, the effect was a simple apnea.

First of all, to throw light upon the origin of this effect, a consideration of

latent intervals will not be out of place. In table 1 are given latent periods for pronounced effects only (the intervals vary somewhat, of course, with the intensity of dose used, rate of injection, etc.), both in the normally innervated animal, and after sinus denervation.

Although the mean values indicate a somewhat shorter latent interval for the effects elicited after sinus denervation, obviously enough such a condition could not in reality exist if both types of injections were made under the same set of circumstances, in view of the fact that the incidence of the more quickly appearing effect, if present both before and after denervation, would determine both latent intervals. It is believed that changes in blood flow brought about by denervation, fluid injections, etc., may account for this slight deviation of one set of latent interval values from the other.

In short, it seems that no sensible difference in latent intervals of effects before and after sinus denervation exists. This in itself would appear to preclude the possibility that the type of effect observed after vagotomy is pulmonary in origin. Furthermore, it is recalled that Nack and Wulp (1929), by ascending catheter injections into the aorta at the level of the bronchial arteries were unable to obtain evidence of a pulmonary seat of action of sulfide. Since the extensive work of Miller on the morphology of the (human) lungs, it is conceded that the terminal capillaries of the bronchial arteries anastomose freely with the respiratory capillaries, and so would carry a chemical into relationship with tissues possessing vagal endings in walls of any type of terminal air passage.

Evidence at hand, then, indicates that effects after sinus denervation are not pulmonary in origin. The latent periods seem to indicate that they are, however, reflex in origin, and arise from a locality supplied at nearly the same instant by arterial blood as that region giving origin to the intense stimulation effect abolished by carotid sinus (and body) denervation. We must bear in mind abdominal regions most directly supplied by aortic blood, as possible seats of this action; but one would less hesitatingly suggest the cardio-aortic zone as the probable responsible zone. This zone appears similarly sensitive to cyanide, where that chemical apparently causes frequently an effect of the same type as that being considered (Winder, Winder, and Gesell, 1933). Heymans and Heymans (1927) have found this zone sensitive, as concerns ventilation, to *physiological* chemical stimuli, also.

That the effect is at least of vagal origin, whatever the particular branch, is demonstrated by the fact that after vagotomy in addition to sinus denervation, doses 3 to 4 times as large either had no effect whatever on respiration, or had an effect resembling those of vertebral injections (fig. 3, K, L, M, N, O, P, Q). The latent periods for these effects after vagotomy (and sinus denervation) were greater than those before by about the same

order of difference as existed between carotid (reflex) effects and vertebral effects, thus further bearing upon the reflex nature of the effect before vagotomy.

A quantity of Na_2S (2.0 to 3.0 cc. of 0.04 M. solution) then, rapidly injected intravenously into normal dogs of the sizes used, comes into relationship with vagal endings (cardio-aortic terminations?) where reflexes are set up which would of themselves cause a retarded laboring respiration. Immediately thereafter, though, the dose passes on into relationship with the carotid sinus region's nerve terminations, where reflexes so much more intense in effect than the first are set up, that they assert themselves almost simultaneously and ordinarily mask completely the first effect, so that the result is an intense transient augmentation of depth of breathing, and usually also of rate. Doses large enough to result in an intense effect of this sort may not be large enough to have any direct central (?) effect whatever.

Depending upon the animal and the intensity of the effect elicited, there may be a post-excitation quiescence of respiratory movements. Possible causes for this quiescence already mentioned are acapnia, and vagus "after-effect" (Head, 1889) resulting from the inspiratory hyperactivity. Now that a stimulating action of Na_2S at the vagal endings has been considered, which of itself retards breathing, it seems that that as an additional possible factor may enter into the quiescent phase, when present.

If very much larger doses be used late action of the chemical at the center may assert itself, which, studied by itself (vertebral injections, or intravenous injections, after sinus denervation and vagotomy), is chiefly a depression (fig. 3, M, P, Q).

FOURTH VENTRICLE INJECTIONS. It was mentioned at the beginning that in a few preliminary experiments (Owen and Gesell, 1931), consistent, marked, and well sustained augmentations of respiratory movements resulted from injections of Na_2S in physiological saline solution directly into the fourth ventricle. In these experiments 0.025 M. or 0.0125 M. solution was used. Eighteen additional experiments have been performed in which a total of 194 injections of Na_2S in saline or Locke's solution, or of H_2S gas, or of H_2S gas dissolved in saline, have been made.

In the first two experiments 0.01 M., 0.02 M., and 0.05 M. solutions of Na_2S in saline were used. Results in these two experiments were so variable, and even diametrically opposed one against the other during one experiment, that uncontrolled extraneous factors were sought for. The intense type of stimulation referred to in the preliminary report (Owen and Gesell, 1931), was frequently represented among these variable results (fig. 4, M).

First of all, frequent control injections of saline eliminated physical

factors and any possible chemical factor inherent in the salt solution itself as originators of the variable results. Occlusion of the common carotid, sinus denervation, or vagotomy had no constant influence on the results.

In a number of subsequent experiments a fresh supply of the Na_2S was used in making the solutions. In these experiments it was found that whether the solution was in ordinary physiological salt solution, Locke's

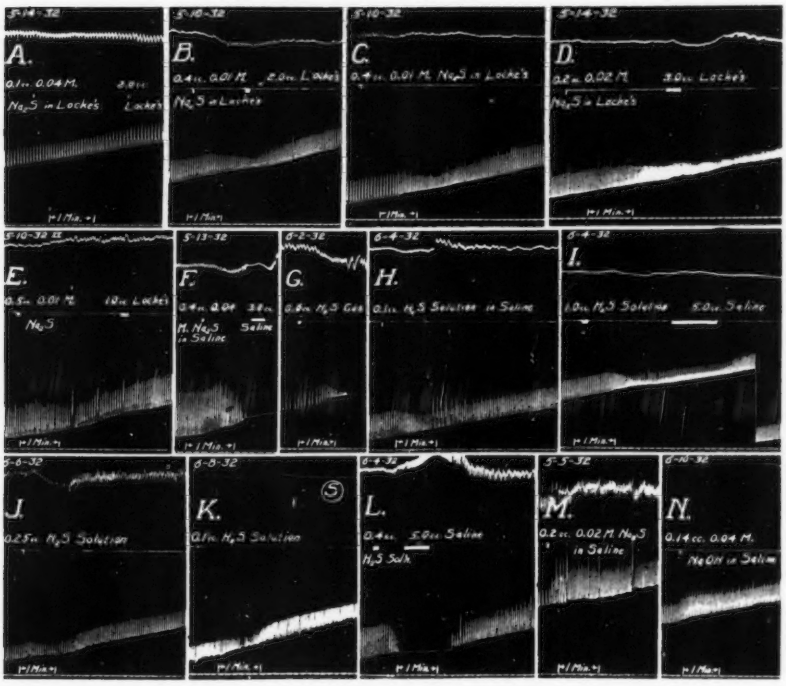

Fig. 4

solution, or Locke's solution without sodium bicarbonate, the results were far more uniform.

In seeking an explanation for the inconstant results obtained before, it was borne in mind that Na_2S is very hygroscopic, and in the presence of water very prone to hydrolysis. The result of hydrolysis is a gradual escape of H_2S, and a residue largely of NaOH, a very strong base. Such might have been the condition of the chemical used in the first experiments.

The results obtained with the new supply of Na_2S were fairly consistently a depression of amplitude, associated either with an increase in rate, a

decrease in rate, or no obvious change in rate. The amplitude depression frequently led to apnea. Except for time relationships, and *frequently a more marked increase in rate*, these effects were similar to those of vertebral artery injections, already discussed.

Even pure Na_2S, however, especially in the presence of tissue buffers, is almost completely hydrolyzed with the liberation of equivalents of NaOH and H_2S; consequently, in order to eliminate the base factor, in many experiments H_2S gas, or its solution in saline, was injected. The results were similar to those with fresh Na_2S solution.

In figure 4 are shown representative effects obtained with the fresh Na_2S, H_2S gas, or H_2S in solution. Each major type of effect shown has been elicited after occlusion of the common carotids in an effort to modify tonic impulses from the carotid sinus region, after vagotomy, after sinus denervation, and after both vagotomy and sinus denervation. Some differences were frequently observed in the results under these various conditions but the differences were inconstant even in direction.

Returning now to the inconsistent results with frequent intense stimulations observed in the first few experiments, and recalling the probability that the difference between these results and the later more constant ones might have been attributable to a deterioration of the chemical used leaving a proportionately large residue of NaOH, it is of interest that injections of NaOH in saline solution, in several experiments, resulted in similar abrupt stimulations (fig. 4, N). The concentration of NaOH was equivalent to that in an hypothetically completely hydrolyzed Na_2S solution of the strength used for these experiments. It seems reasonable to assume that the base present in old "Na_2S" may either have been entirely responsible for intense stimulations obtained with it, or that the base might have so modified the action of H_2S on the tissues as to have resulted in these stimulations.

SUMMARY

In dogs anesthetized with morphine and urethane, abrupt, intense transient augmentations of pulmonary ventilation, especially the depth thereof, were consistently observed on injection of Na_2S into the common carotid with normally innervated sinus region. The average minimal dose required for an effect contained 0.0006 mgm. of Na_2S per kgm. body weight. The response with its very short latent interval and the minute order of dose required to elicit it, is quite similar to the corresponding response to sodium cyanide. Heymans et al.'s report and Owen and Gesell's preliminary report of such a response are thus confirmed. In further agreement with these reports, the intense response is eliminated by carotid sinus (and body) denervation.

A similar abrupt response is consistently elicitable by rapid femoral intravenous injections of sulfide.

After sinus denervation an intravenous dose of the same order elicits an altogether different type of response, consisting usually of a slowed, laboring breathing. The latent interval remains sensibly the same as that for the effect before sinus denervation.

After vagotomy in addition to sinus denervation, doses two or three times as large usually have no effect on respiration, thus demonstrating that the effect after sinus denervation is also of reflex origin; viz., vagal. Reasons are given for believing that the cardio-aortic branch rather than pulmonary is involved. The reflex is less intense than the carotid reflex, and more in the nature of an inhibition than intense excitation.

When sufficiently large doses are intravenously injected after sinus denervation and vagotomy, effects resembling those of vertebral artery injections are obtained. The latent period is longer than that for the reflex effects.

Vertebral intra-arterial injections elicit effects on respiration after a definitely longer latent interval than that for normal carotid injection effects. If near minimal, these vertebral effects may be a very slight and transient simple increase in rate, or rate and depth of breathing, frequently associated with more or less increase in mean pulmonary volume. A moderate effect is characterized by a depression of depth of breathing, accompanied by a slowing, no change, or occasionally an increase in rate. Preceding the depression there may or may not be a slight and short-lived increase in rate, amplitude, or tonus, or any two or all three of these. Overshooting recoveries from the depression may occur, but seldom if ever in a degree to save the whole effect from being a reduction in volume exchange of gases.

Large doses accentuate these phases, especially the depression, which may lead to apnea of varying duration. Permanent damage to or failure of respiration may result from large doses. There are reasons for believing this vertebral action to be directly on brain-stem nuclei concerned with respiratory movements.

Direct injections into the fourth ventricle of fresh Na_2S in saline solution or Locke's solution, or of H_2S gas, or of H_2S in saline solution, elicit responses of respiratory movements similar to those of vertebral artery injections, excepting time relationships, and frequently more marked increases in rate in the case of the former.

REFERENCES

GAY, H. 1930. This Journal, xcv, 519.
HAGGARD, H. W. 1921. Journ. Biol. Chem., xlix, 519.
HAGGARD, H. W. AND Y. HENDERSON. 1922. This Journal, lxi, 289.

HEAD, H. 1889. Journ. Physiol., x, 1.
HERING, H. E. 1927. Die Karotissinusreflexe auf Herz und Gefässe. Dresden und Leipzig.
HEYMANS, C. AND J. J. BOUCKAERT. 1930. Compt. rend. Soc. de Biol., ciii, 498.
HEYMANS, C., J. J. BOUCKAERT AND L. DAUTREBANDE. 1930. Compt. rend. Soc. de Biol., cv, 881.
1930. Arch. internat. de Pharm. et de Thérap., xxxix, 400.
1931. Compt. rend. Soc. de Biol., cvi, 52.
1931. Arch. internat. de Pharm. et de Thérap., xl, 54.
HEYMANS, J. F. AND C. HEYMANS. 1927. Arch. internat. de Pharm. et de Thérap., xxxiii, 273.
NACK, A. AND G. WULP. 1929. This Journal, lxxxix, 315.
OWEN, H. AND R. GESELL. 1931. Proc. Soc. Exp. Biol. and Med., xxviii, 765.
WINDER, C. V., H. O. WINDER AND R. GESELL. 1933. This Journal, cv, 311.

THE SENSITIVITY OF THE STRIATED MUSCLE TO CHANGES IN pH

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Received for publication May 18, 1933

Although there is a great deal of information on the effect of changes in pH on the function of heart, smooth muscle and other tissues (compare Haffner (1927) and Gellhorn (1931) for further information), our knowledge in regard to the sensitivity of the striated muscle in this respect is very meagre. Neugarten (1919) showed that significant differences in excitability and muscular work exist between muscles immersed in neutral solution (pH 7–7.8) and acid and alkaline solutions of pH 4.5 and 9.5 respectively. An attempt to determine the reactivity of the striated muscle to slighter changes of pH was made by Grant (1920), who found he could just detect an increase in excitability at pH 9 and a decrease at pH 4 to 5, whereas solutions with a smaller deviation from neutrality seemed to have no effect. An objection must be raised against these experiments on the ground that phosphate solutions of different pH were compared with saline solutions and therefore the ionic composition of the two solutions to be compared was quite different. Berg (1911) described a beneficial effect of $NaHCO_3$ on fatigued muscle, but here again no experiments were carried out in order to determine systematically the influence of solutions of different pH on the striated muscle.

If this earlier work would represent a true picture of the striated muscle in regard to its sensitivity to alterations of the pH, it would have to be assumed that it reacts essentially differently from smooth muscle. The latter shows a great sensitivity to variations in pH since Fleisch (1921) has shown in his work on the influence of pH on the diameter of blood vessels that a change in [H] from 0.35×10^{-7} to 0.7×10^{-7} (i.e., from pH 7.52 to 7.15) is sufficient to alter significantly the diameter of the blood vessels. In a very recent paper (1932) Fleisch shows with an improved method that a solution which is only 0.17 pH unit more acid than normal blood leads to a considerable increase in the perfusion rate in cats.

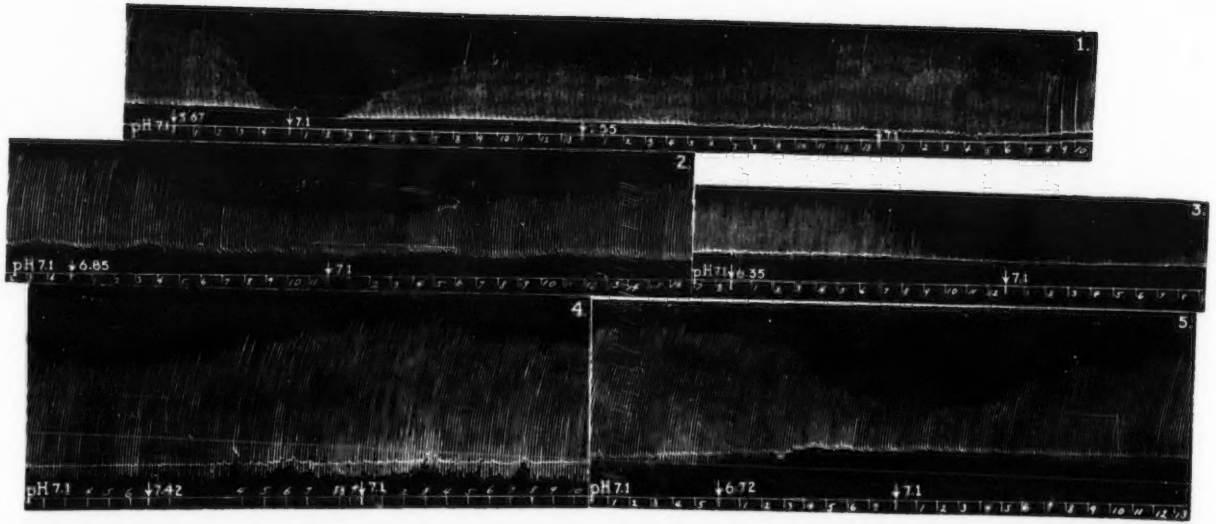
[1] Aided by a grant of the National Research Council.

The present study of the influence of pH upon the striated muscle was mainly undertaken to investigate the question of whether essential differences exist in this regard between striated and smooth muscle. This seemed the more necessary since other cations (alkali metals) influence both muscles in a similar manner (Gellhorn, 1923). Furthermore an exact knowledge of this factor seemed necessary in other studies on muscular fatigue which are being carried out by the author.

Previous studies on ion effects in fatigued muscles (Gellhorn, 1931–1932) seem to indicate that under these conditions the muscles are more susceptible to ionic influences than are fresh muscles. Moreover, it seemed likely that if the muscles would be perfused with different solutions the effects might be more distinct than in immersion experiments. This seems to be indicated by Fleisch's experiments in contrast to observations of Krogh (1929), who, using the application of acids from the outside, was unable to detect such delicate reactions of the blood vessels as were observed by Fleisch. For this reason the reactivity of the fatigued muscle was studied during perfusion with solutions of different pH.

Method. Frogs (Rana esculenta) were pithed and halved by a horizontal cut through the lumbar region. After removal of the abdominal organs a cannula was inserted into the abdominal aorta and the hind legs were perfused. By means of several three-way stopcocks the hind legs could be perfused with different solutions. The knees were fixed on the frog board with needles and the board was placed in a vertical position. The tendon of one gastrocnemius was cut free and attached to an isotonic lever. The muscle was stimulated indirectly by maximal break shocks of a Harvard induction coil, the make shocks being short circuited. At first the muscle was fatigued by stimulating the nerve at a frequency of about 40 per minute. After the height of contraction was about 50 per cent of the original we changed to a low frequency (about 10 per minute) and at this frequency exchanged solutions with various pH. They consisted of 90 parts Ringer solution without $NaHCO_3$ (NaCl 6.0, KCl 0.075, $CaCl_2$ 0.2 in 1 liter H_2O) plus 10 parts phosphate buffer. The latter was composed of $\frac{m}{10}$ Na_2HPO_4 and $\frac{m}{10}$ NaH_2PO_4 in various proportions. The pH of all solutions was electrometrically determined. Under the conditions of our experiments the phosphate concentration was constant and only slight variations in Na occurred due to the different mixtures of Na_2HPO_4 and NaH_2PO_4. They are, as control experiments show, too small to show any effect.

Results. The outstanding result of our experiments is the fact that the striated muscle in a state of relative fatigue (Asher 1927) when perfused with a phosphate buffered Ringer solution reacts in a very sensitive way to slight changes in pH. In all experiments a solution of pH 7.1 was used as



Figs. 1–5. Perfusion experiments in Rana esculenta. Indirect stimulation with maximal break shocks. Numbers represent time in minutes. Ringer-phosphate solutions.

the standard and the effects of solutions with a higher or lower pH were compared with the contractions obtained in the standard perfusion experiments. Figure 1 shows that a slight increase in the acidity (pH = 5.67) leads to a complete disappearance of the contractions. The first effect occurs after 2 minutes, which is simply due to the fact that according to the perfusion rate the solution reaches the preparation after from 2 to 4 minutes after the stopcock has been turned. In accordance with this fact we see that recovery sets in in Ringer of pH = 7.1 at the end of the second minute. The recovery may be considered as complete, although the height of contraction is somewhat lower than it was at the beginning of the experiment. But it must be taken into account that the preparation shows some decline in the standard solution, as the first part of the curve in figure 1 indicates. After a somewhat steady state has been reached in the standard solution, the muscle is perfused with a more alkaline solution (7.55) and thereafter with standard Ringer of pH 7.1. It is quite obvious that the height of contraction increases distinctly in the more alkaline solution. Although the deviation of the acid and of the alkaline solution from the pH of the standard solution is the same, it is quite obvious that the changes toward the acid side are more pronounced. This is quite typical, as the other figures show. Figures 2, 3 and 5 give some more information in regard to the effect of acid Ringer solutions. Even by a solution of pH 6.35 the contraction can be suppressed almost completely, but the lowering of the height of contraction is brought about more slowly, as figure 3 indicates. Solutions with a still slighter deviation of the pH to the acid side are reproduced in figures 2 and 5. In both cases (pH 6.85 and 6.72) the contractions are diminished but not suppressed. It is noteworthy that a difference of 0.25 pH unit, as figure 2 shows, alters the excitability of the muscle considerably. It is very probable that even smaller differences in pH would influence the excitability of the fatigued muscle. The experiment of figure 2 and a number of similar observations which have always led to the same result indicate quite plainly the sensitiveness of the striated muscle to slight changes in pH. *It must be stated on the basis of the experiments described in this paper that the sensitivity of striated and smooth muscle in regard to pH is of the same order.*

It has already been mentioned that a change from pH 7.1 to 7.55 increases the height of contraction of striated muscles (fig. 1). Although the differences are slighter than are found on the acid side for an equal difference in pH, such changes are still quite obvious on the alkaline side with a change in pH from 7.1 to 7.42, as shown in figure 3.

Since maximal stimuli were used, the data must be interpreted as indicating a change in reactivity of the individual muscle fibers rather than a change in a number of fibers which contract.

SUMMARY

The influence of changes in pH in Ringer solutions containing phosphate buffers on the striated muscle was studied in perfusion experiments (Rana esculenta). The solutions contained the same amount of phosphate and varied only slightly in their Na content, which was found to be without effect on the excitability of the muscles. Therefore the differences refer exclusively to the pH of the solutions. It was found that pH differences of 0.25 unit distinctly alter the response of the muscle to indirect stimulation with faradic break shocks. Acid solutions decrease and alkaline solutions increase the height of contraction. The changes are reversible within the limits (pH 5.6 and 7.6) which were studied in this investigation. It is therefore obvious that in contrast to the previous reports no significant differences exist between striated and smooth muscle in regard to pH. Earlier studies were probably in error because the muscles were simply immersed in solutions of several pH values, whereas perfusion of the muscle through its vessels is absolutely essential if reliable results are to be obtained. It is also to be noted that more pronounced effects may be seen in the fatigued than in the fresh muscle.

REFERENCES

ASHER, L. 1927. Naturwissenschaften, xv, 391.
BERG, F. 1911. Skand. Arch. f. Physiol., xxiv, 345.
FLEISCH, A. 1921. Zeitschr. f. allg. Physiol., xix, 270.
FLEISCH, A., I. SIBUL AND V. PONOMAREV. 1932. Pflüger's Arch., ccxxx, 814.
GELLHORN, E. 1923. Pflüger's Arch., cc, 583.
1931. Biol. Bull., lx, 382.
1932. This Journal, c, 447, 452.
1931. Lehrbuch der allgemeinen Physiologie. Leipzig, p. 98.
GRANT, M. H. 1920. Journ. physiol., liv, 79.
HAFFNER, F. 1927. Handb. d. exp. Pharm., Berlin. Vol. iii, part I.
KROGH, A. 1929. Anatomie und Physiologie der Capillaren. Berlin.
NEUGARTEN, T. 1919. Pflüger's Arch., clxxv, 94.

THE EFFECT OF CERTAIN CALCIUM SALTS ON THE RHYTHMICALLY CONTRACTING AND QUIESCENT UTERINE FISTULA, WITH OBSERVATIONS ON THE ACTION OF POSTERIOR PITUITARY EXTRACTS

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Received for publication May 15, 1933

The importance of the calcium ion for the normal irritability of the excised guinea-pig uterus has been investigated and the essentiality of this ion for sensitivity to pituitary extracts clearly established (van Dyke and Hastings, 1927–28). There is less certainty, however, that the uterus *in vivo* is similarly dependent upon the calcium environment of the blood or that uterine contractions are affected by calcium administration. Wodon (1931) claims that the contracting uterus of the anesthetized guinea pig is caused to relax as a consequence of administration of the calcium or magnesium ion. The latter ion, according to Wodon, is more effective in this regard than is the former. Weinstein and Friedman (quoted by Friedman and Friedman, 1933), on the other hand, find that the contracting uterine fistula of the rabbit is unaffected as a result of intravenous administration of calcium (calcium gluconate).

The data of the present paper consist of observations on the effect of calcium chloride and calcium gluconate (Calglucon, Sandoz; 1 cc. 10 per cent solution = 9.3 mgm. calcium) on the quiescent and contracting uterus of the unanesthetized rabbit, with a correlation of the latent period and duration of the response—when it occurs—with the rate and route of administration. In addition, the responsiveness of the quiescent and contracting uterus to posterior pituitary extracts following calcium administration has been studied and also a few observations on the effect of magnesium chloride have been made.

Following the method previously reported (Reynolds, 1930a; Reynolds and Friedman, 1930), we have employed the uterine fistula technic of recording uterine contractions by means of an intra-uterine balloon in chronic experiments on the unanesthetized rabbit. While we realize that the rabbit is not the most suitable animal for calcium studies (Thomson and Collip, 1932), nevertheless when due allowance is made for conditions which affect the calcium level of the blood, this animal may be employed

successfully (Hogben and Charles, 1932). In the present work, however, the level of calcium and its distribution in the blood have not been followed; rather, the effects observed have been correlated with known amounts given intravenously or subcutaneously. Thus the approach to the problem is only qualitative.

In work of this sort it is necessary to employ a "standard" rabbit inasmuch as the sexual state of the animal profoundly affects both the motility of the uterus and its responsiveness to drugs. Accordingly, untreated castrated does were employed when the response of the quiescent[1] uterus was to be investigated, but when observations on a contracting uterus were desired a single subcutaneous injection (25–50 r.u.) of oestrin was given eighteen to twenty-four hours prior to the experiment (Reynolds, 1933).

Results. The present results consist of fifty-eight separate observations of the effect of calcium therapy in eight rabbits on thirty-one different days. In addition numerous observations were made on the modifications of the calcium response of the uterus following injection of the pressor principle of the posterior pituitary body. A typical protocol of one of the rabbits used in these studies is given below.

Rabbit 5. Weight, 2.7 kgm.

1/26/33 Operated; castrated and uterine fistula prepared.
Ovaries contained medium-large follicles.

2/ 2/33 25 r.u. Theelol, subcutaneously.

2/ 3/33 Motility ++‴+; rhythmical, frequency, 45″–60″.
5:20 p.m., 12 cc. calcium gluconate intravenously, over a 4′ period. Response: sustained contraction lasting 5–6 minutes followed by elevation of tone and partially summated contractions lasting ½ hour, then discrete contractions of more rapid frequency than pre-injection motility.
8:50 p.m., gastro-intestinal distress, and uterine fistula retracted. Motility ++; frequency 60″–90″.
25 r.u. Theelol, subcutaneously.

[1] In a recent dissertation by Dr. A. W. M. Pompen on "De Invloed van Menformon op der Baermoeder" exception is taken to the term "quiescent" as used by the present writer. Pompen, observing the uterus through an abdominal window, finds that under the conditions described as "quiescence" when recorded by an intra-uterine balloon, there are in reality feeble contractions of an incoördinate character which are local; on the other hand, when oestral contractions of a marked type have been described, Pompen concurs that they do exist and that these are contractions in which the whole uterus is involved. It is not difficult to see why we have spoken of "quiescent" or "relatively quiescent" uteri when feeble, local contractions occur, inasmuch as these could only by chance cause some diminution in volume of the intra-uterine balloon, and hence be recorded as contractions. From the practical standpoint, however, of *effective action* upon a bolus contained within the viscus the uterus as a whole is indeed quiescent. Such a conception facilitates an appreciation of the factors that permit continuance of gestation until the onset of labor, when the uterine musculature is somewhat irritable and shows local muscular activity. From a physiological standpoint, however, an understanding of the coördinated and uncoördinated uterine movements is desirable.

2/ 4/33 Motility +++; rhythmical, frequency 45″-60″.
2:00 p.m., 3 cc. calcium gluconate injected intravenously over 20″. Response: after 30″ a sustained contraction lasting 2′ followed by frequent sustained contractions lasting 40″–60″ each. Approximately normal motility in ¾ hour. 25 r.u. Theelol, subcutaneously.

2/ 5/33 Motility +++; rhythmical, frequency 45″–60″.
3:00 p.m., 1.5 cc. calcium gluconate, injected rapidly. Response: 30″ after commencement of injection, a partially summated contraction occurred lasting 2′, followed by others also partially summated. Frequency increased, 30″.
3:20 p.m., 4 cc. calcium gluconate. Followed in 30″ by partially summated contraction lasting 2′, then frequency of contractions increased. No increase in amplitude of contractions. No Theelol.

2/ 6/33 Motility ++-+++; arrhythmical (typical waning of oestrin). 3:15 p.m., 1.0 cc. calcium gluconate, injected rapidly. Response: a feeble summated contraction, lasting 1′, then diminished activity for 5′.
3:25 p.m., 4.0 cc. calcium gluconate intravenously, rapidly. Response: 35″ later a summated contraction lasting 1′, then diminished motility for 10′.

2/11/33 Motility, 0 (no oestrin for one week). Figure 1, A.
7 cc. calcium gluconate intravenously in 30″. Response: nil (see fig. 1, A *1*). Eight minutes later 1 cc. oxytocin (Pitocin) intravenously. A feeble indication of a sustained contraction (fig. 1, A *2*) (cf. Reynolds, 1930b). 25 r.u. Theelol, subcutaneously.

2/12/33 Motility +++; rhythmical, frequency, 35″.
9:20 a.m., 1.5 cc. calcium gluconate, intravenously, in 30″ (see fig. 1, B *1*). Response: sustained contraction lasting 2′.
9:31 a.m., 1.0 cc. calcium gluconate (fig. 1, B *2*). Response: a 2′ sustained contraction.
9:36 a.m., 10.0 cc. calcium gluconate, moderate venous infusion (fig. 1, B *3*) injected over 8′. Series of sustained contractions resulted, outlasting injection period. 0.5 cc. pitressin (Parke, Davis & Co.) at B *4*. Followed in 4½′ by profound diminution of motility. 4 cc. calcium gluconate (B *5*): no response. 1.0 cc. pitocin (b *6*): no response. Large mechanical response by applying pressure to abdomen (M).

2/13/33 Motility +++; rhythmical, frequency, 30″–35″.
1:15 p.m., 0.5 cc. calcium gluconate, slow venous infusion (½ cc./min.) Response: 1′ later, a sustained contraction lasting 3′ followed at once by another contraction lasting 2′.
1:28 p.m., 3 cc. calcium gluconate, moderate venous infusion (1 cc./min.) Response: latent period of 1′, then succession of sustained contractions for 15′ (two longest, 5′ each).
1:50 p.m., 2 cc. calcium gluconate, rapid injection (4 cc./min.) Response: in 30″ a long sustained contraction resulted which was caused to be inhibited after 3′ by injection of 0.5 cc. pitressin. Uterus was then refractory to 1 cc. pitocin and 4 cc. calcium gluconate.

There are several aspects of the above protocol which deserve comment and which are borne out by the protocols obtained from the remaining seven rabbits. These, along with other pertinent observations, are briefly discussed below.

1. *Dependence of calcium response upon pre-injection motility.* (Table 1; fig. 1, A and B.) From the data summarized in table 1 it is apparent that if an unmistakable response (sustained contraction or series of such) is to result from the injection of calcium salts, the uterus must be rhythmically contracting prior to the injection. It is true that in five of twenty-four instances in which feeble pre-injection motility was observed, slight calcium responses did result, but these responses were no more than feeble contractions or elevations of tone which are not reliably obtained under such circumstances. The data of this table are in accord with what one finds for the response of the uterine fistula to pitocin and pituitrin (Reynolds, 1930b), and for an anaphylactic contraction of the uterus (Weinstein, Reynolds and Friedman, 1931). Such rhythmical contractions as appear to be essential for these responses seem to be dependent upon one class of substances, namely, oestrogenic ones (Reynolds, 1931, 1933a).

TABLE 1

Dependence of the calcium response of the uterus upon pre-injection motility

PRE-INJECTION MOTILITY	INSTANCES SHOWING		TOTAL
	Calcium chloride or gluconate response	No calcium salt response	
Marked (+++)	22 (0.5–10 cc. i.v.) (15–20 cc. s.c.)	0	22
Moderate (++)	8 (more than 2 cc.)	4 (0.5–1 cc.)	12
Minimal or (+–0) quiescent	5 (3–6 cc.)	19 (1–10 cc. i.v.) (15–20 cc. s.c.)	24
Total	35	23	58

i.v.—intravenous; s.c.—subcutaneous.

2. *Effect of rate of administration of calcium: on latent period.* One of the characteristics of the calcium response which is affected by the rate at which the salt is administered is the onset of the sustained contraction, timed from the first moment of the start of the injection. When the intravenous injection rate is rapid (2 cc. or more per minute) the latent period is regularly thirty to thirty-five seconds as compared with five seconds for a response to pituitary extracts and ninety seconds for an anaphylactic response.

When the injection rate is moderate or slow ($\frac{1}{2}$-1 cc. per minute) however, the latent period is considerably lengthened. The latent period of the onset of the sustained contraction falls well within the second minute instead of the first half-minute, and when the subcutaneous route is used the latent period is still longer (4–6 minutes) as indicated in figure 2.

On duration of the calcium response. It is impossible to answer for a

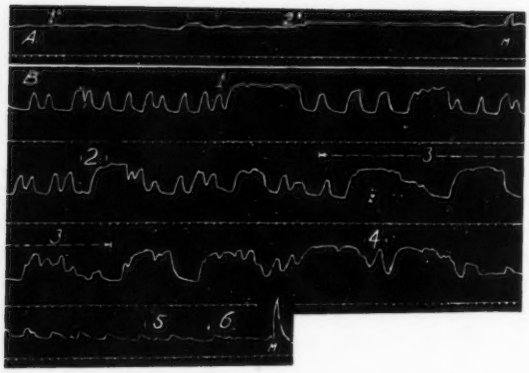

Fig. 1. Records showing the effect of pre-injection motility in determining the response of the uterine fistula to the injection of calcium gluconate.

Rabbit 5 (see protocol). A, 2/11/33. Quiescent uterine fistula of castrated rabbit. *1*, 7 cc. calcium gluconate; *2*, 1 cc. pitocin. *M*, mechanical response obtained by pressing abdomen.

B, 2/12/33. Twenty-four hours after Theelin (to induce rhythmic motility). *1*, 1.5 cc. calcium gluconate; *2*, 1.0 cc. calcium gluconate; *3*, slow venous infusion of 10 cc. calcium gluconate; *4*, 0.5 cc. pitressin; *5*, 4 cc. calcium gluconate; *6*, 1.0 cc. pitocin. $\frac{1}{10}$ original size.

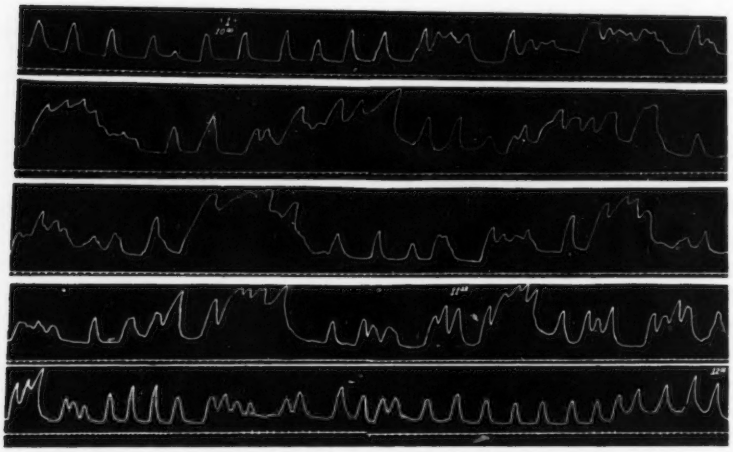

Fig. 2. Continuous records showing effect of subcutaneous injection of calcium gluconate (20 cc.). Note long (6 minute) latent period of response. $\frac{1}{10}$ original size.

certainty from our data the question as to what factor may be the major one determining the duration of the calcium response. Several considerations point to the fact however that the rate at which the calcium is administered as being an important factor.

For example, when large amounts of calcium salts (five to eight cubic centimeters) are injected in the space of a few seconds, the sustained contractions which follow are not regularly of greater duration than are the contractions which follow the rapid injection of smaller amounts ($\frac{1}{2}$-1 cc.) provided sufficient calcium is injected to elicit any response whatsoever. Still another consideration points to the importance of the effect of rate, rather than total amount injected as major factor in this respect. When the injections are made at moderate or slow rates a succession of sustained contractions ensues which far outlast the contractions which result from similar or larger amounts of calcium rapidly administered. Yet a third point may be mentioned in this regard. It is well established that the highest level of calcium in the blood following administration by the oral, intramuscular (man and dog, Lieberman, 1931) or subcutaneous route (rabbit, Rothlin, 1929) lies within the second hour, whereas the period of greatest activity of the uterus occurs well within the first hour after subcutaneous administration of calcium gluconate (see fig. 2). From consideration of the above points, it seems clear that the relationship between rate of administration and the duration of the response is a direct one,—a prolonged injection span favoring a more prolonged response. The reason for this is not clear, however.

3. *Calcium chloride and calcium gluconate.* Of the fifty-eight instances in which injections of calcium alone were administered twelve of these were with calcium chloride, forty-six were with calcium gluconate. With each salt the concentration of calcium approximated 10 mgm. per cubic centimeter of solution. Under none of the circumstances observed was the respective action of these two salts dissimilar as regards the characteristics enumerated above. As reported elsewhere however (Rothlin, 1929; see also Szurek, 1930), it was found that calcium chloride is not tolerated when given subcutaneously without causing local necrosis. Such is not the case with calcium gluconate.

4. *Action of pitressin on calcium and pitocin responses* (fig. 1 B, *4*, *5*, *6*). The action of pitressin was investigated, to determine whether or not the sustained contractions resulting from calcium administration might be inhibited as may be the action of pitocin in the period of quiescence which follows the contraction of the uterine fistula under the influence of whole pituitrin (Weinstein and Friedman, quoted by Friedman and Friedman, 1933; pituitrin and pitocin responses, Reynolds, 1930b). The action of pitressin is illustrated in fig. 1 B, *4*. Three to four minutes after the injection of pitressin the contracted uterus becomes relatively quiescent and is

absolutely refractory to subsequent injections of calcium or pitocin unless the uterus is again motile. Moreover, it was frequently observed that pitressin abolishes for a time the spontaneous contractions of the uterus and renders this organ transiently refractory to both calcium and pitocin.[2]

5. *The effect of magnesium chloride.* Sufficient experiments (ten in number) have been performed to warrant certain statements relating to the effect of magnesium chloride on the uterine fistula. With small or moderate intravenous doses (0.5–1 cc., containing 10 mgm. of magnesium per cubic centimeter) no discernible effect may be seen when the uterus is spontaneously contracting. When, on the other hand, the uterus is contracted under the influence of calcium, magnesium brings about an immediate relaxation with a resumption of spontaneous contractions. Injections of magnesium also prevent a subsequent calcium response. The present results do not agree with those reported by Wodon (1931) who finds relaxation of the uterus of the anesthetized guinea pig following both magnesium and calcium therapy.

Discussion. As mentioned at the outset, van Dyke and Hastings (1927–28) demonstrated the essentiality of the calcium ion for a posterior pituitary response of the quiescent uterus *in vitro*. In the present work it has been found that pitocin is not effective on the quiescent uterus either before or after calcium administration; sometimes the virtually quiescent uterus does seem to give a feeble response (fig. 1 A, *2*), but this does not seem to be more pronounced after calcium therapy than it is before. The present observations do not vitiate the point clearly established by van Dyke and Hastings however, that at times when the posterior pituitary extracts are able to elicit a response such a response is in some way dependent upon the presence of calcium.

In conclusion a possible relationship between the results outlined above and a recent recommended use of calcium therapy may be mentioned. Bardenheuer (1929) finds that *post partum* hemorrhage may be effectively diminished by the use of calcium administered intramuscularly whereas administered intravenously, it is relatively ineffective. In the light of the present observations we venture to suggest that part of the hemostatic action of calcium therapy following delivery might possibly find explana-

[2] Robson and Illingworth (1932, p. 94) impute to the writer the observation that pitressin exerts an action on the uterus similar to that of pitocin. Up to the present time the action of pitressin on the uterine fistula has not been reported. Frequently, though by no means always, the injection of pitressin causes a sustained contraction which gives way to relative quiescence and absolute refractoriness to further stimulation for some minutes. This is attributed to contamination of the pressor extract with small amounts of the oxytocic factor, inasmuch as the response is indistinguishable from that elicited by whole pituitrin. The converse, however, of quiescence following an oxytocic response elicited by pitocin has never been observed either before (Reynolds, 1930b) or now.

tion in the oxytocic action of calcium as described above, provided of course it can be demonstrated that the human uterus *in vivo* is affected by calcium therapy in a manner similar to that of the rabbit. Experiments alone can establish this point.

SUMMARY

Calcium chloride and calcium gluconate in equivalent calcium concentrations have identical effects upon the uterine fistula of the unanesthetized rabbit. These effects are as follows: 1. Sustained contractions in response to calcium injection result only when the uterus is rhythmically contracting (as determined by the intra-uterine balloon method). 2. The latent period of response seems to be dependent primarily upon the rate of administration of calcium (30–35 seconds when administered rapidly, 60–120 seconds when administered slowly by the intravenous route; 4–6 minutes when administered subcutaneously). 3. The duration of the sustained contractions seems to be determined not so much by the total amount of calcium injected as the span of the injection time. 4. Pitressin (pressor posterior pituitary extracts) inhibits sustained contractions induced by calcium; inhibits rhythmical uterine motility, and renders the uterine fistula transiently refractory to further injections of calcium or pitocin (oxytocic posterior pituitary extract). 5. Magnesium chloride inhibits a sustained contraction induced by calcium and transiently prevents a further calcium response; in small amounts however (5–10 mgm. magnesium) it exerts no discernible effect upon spontaneous, rhythmic uterine motility.

REFERENCES

BARDENHEUER, F. H. 1929. Zentral. f. Gynäk., liii, 1826.
FRIEDMAN, G. S. AND M. H. FRIEDMAN. 1933. This Journal, ciii, 244.
HOGBEN, L. AND E. CHARLES. 1932. Journ. Exp. Biol., ix, 139.
LIEBERMAN, A. L. 1931. Journ. Pharm. Exp. Therap., xlii, 245.
POMPEN, A. W. M. 1933. Dissertation, Amsterdam.
REYNOLDS, S. R. M. 1930a. This Journal, xcii, 420.
1930b. This Journal, xcii, 430.
1931. This Journal, xcvii, 706.
1933. Proc. Soc. Exp. Biol. Med., (in press).
REYNOLDS, S. R. M. AND M. H. FRIEDMAN. 1930. This Journal, xciv, 696.
ROBSON, J. M. AND R. E. ILLINGWORTH. 1932. Quart. Journ. Exp. Physiol., xxi, 93.
ROTHLIN, E. 1929. Zeitschr. f.d. ges. exp. Med., lxx, 1.
SZUREK, S. A. 1929. Proc. Soc. Exp. Biol. Med., xxvi, 773.
THOMPSON, D. L. AND J. B. COLLIP. 1932. Physiol. Rev., xii, 309.
VAN DYKE, H. B. AND A. B. HASTINGS. 1927–28. This Journal, lxxxiii, 563.
WEINSTEIN, G. L., S. R. M., REYNOLDS AND M. H. FRIEDMAN. 1931. This Journal, xcviii, 237.
WODON, J. L. 1931. Arch. Int. Med. Exper., vi, 1.

A METHOD OF STIMULATING AUTONOMIC NERVES IN THE UNANESTHETIZED CAT WITH OBSERVATIONS ON THE MOTOR AND SENSORY EFFECTS

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From the Laboratories of Physiology in the Harvard Medical School

Received for publication May 8, 1933

In the course of investigations of the autonomic nervous system conducted in this Laboratory, it was suggested that a method for stimulation of the efferent nerves of this system in the unanesthetized animal might be valuable. For this purpose electrodes, described below, were devised. They have been proved to be readily applicable to nerve trunks in the cat, they have been left in place for weeks without disturbance of the animal, and have been stimulated at will.

The practicability of using more or less permanently implanted electrodes for the purpose just mentioned depends on the absence of pain fibers in the nerves. Evidence on that point is fairly concordant for some regions of the body but not for others. Thus physiological experiments (Langley, 1900) and also anatomical studies (Ranson and Billingsley, 1918) have led to the statement that "evidence is against the presence of afferent fibers in the cervical portion of the sympathetic trunk." Davis and Pollock (1932), and Cleveland (1932), who stimulated the cervical sympathetic trunk below the superior cervical ganglion in a lightly or non-anesthetized cat, have recently confirmed this conclusion. On the other hand, Foerster, Altenberger and Kroll (1929) have reported that faradic stimulation of the caudal end of the cut cervical sympathetic causes pain. The presence of pain fibers in the splanchnics has long been known; indeed, Foerster, Altenberger and Kroll regard them as the most important pain nerves of the viscera, though not the only pathway of the pain-carrying fibers. Kappis (1920) found visceral sensory fibers not only in the splanchnics but also in the *rami communicantes* of the first three lumbar ganglia.

Most of the evidence concerning pain fibers in the sympathetic supply to the limbs has come from studies on the sensitivity of blood vessels. Friedrich (1924), Schilf and Stahl (1925) and Abrashanow (1927) have all reported that after severance of the femoral and sciatic nerves in the dog the leg arteries are still sensitive, and mediation by sympathetic fibers is indicated by the disappearance of the phenomenon after periarterial "sympathectomy." In opposition to these observations are those of Moore and

Singleton (1933), who found that section of the femoral and sciatic nerves does not deafferent a hind limb, that the posterior femoral cutaneous nerve still functions, that when in addition that nerve is cut all signs of pain conduction disappear, and that section of the posterior roots from L5 to S3 inclusive likewise abolishes the pain reflexes. The pain fibers, they conclude, do not traverse the sympathetic chains.

Lennander (1906) and Foerster (1927) are in agreement in declaring that below the junction of the recurrent branch the vagi carry no pain impulses. Lebendenko and Brjussowa (1930), however, observed changes on distending the stomach, after severing all other nerve connections except the vagi, which led them to ascribe to the vagus the possibility of conducting pain impulses. And Spiegel (1927) likewise has expressed the judgment that the vagus nerves share in pain conduction from the aorta. A participation in the pain of gastric crises has also been ascribed to the vagi (see Shawe, 1922).

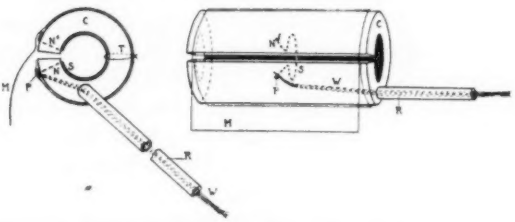

Fig. 1. Schematic drawing of the shielded electrode for implantation. C = catheter tube; M = rubber membrane; N and N' = pinholes; P = solder point; R = rubber tubing; S = silver wire; T = silk thread suture; W = enameled copper wire.

From the foregoing review it is clear that opinions regarding conduction of pain impulses in autonomic courses are not unanimous. Foerster, Altenberger and Kroll (1929) go so far as to testify that there is no part of the body in which sensory fibers (the context implies pain fibers) of the sympathetic do not play a rôle. In opposition, other observers report evidence which does not support this conclusion. Previous studies, however, have been made under local or general anesthesia. Implanted electrodes offer opportunity to examine, with great delicacy, the results of sympathetic and vagal stimulation in the normal unanesthetized animal.

Method. The electrodes used in making the observations, and shown diagrammatically in figure 1, were constructed as follows. A segment about 1 cm. long is cut from a soft rubber catheter, C, no. 8 French, to form the wall. The tube is split longitudinally and at the middle, about 0.5 mm. to either side of the split, two pinholes N and N' are made. Into one of these a fine cutting needle is pushed and directed lengthwise through the

wall of the tube until it emerges at the end. Seven or eight strands of enameled copper wire, *W*, no. 33 or 34, threaded into the eye of the needle, are then drawn through the rubber by means of it.

A soft silver wire, *S*, no. 27, is now passed through the two pinholes and fitted to the round lumen of the catheter wall. To fasten the wire in place it is sutured with silk thread at point *T* and bent back on itself at pinhole *N'*. At pinhole *N* the wire is left protruding about 2 mm. The free ends of the copper wires, with the enamel scraped off, are wound about the silver wire at this point, *P*. All ends are trimmed down as closely as possible; a drop of solder placed on the junction insures a good electrical and mechanical connection. The excess soldering paste and any other dirt are washed off with ether.

In order to insulate the tissues that are to lie around the electrode thin rubber membrane, *M*, is fastened over its surface with ordinary tire cement. This covers the exposed wires, but, as an added precaution, a flap is left to be slipped around the electrode after it has been implanted.

As the activity of the muscles through which the enameled wires pass may occasionally chip off the insulation or actually break the wires, it has been found wise to cover them with soft white rubber tubing, *R*, 1 mm. inside diameter. The tubing is held by rubber cement in the position shown in the diagram (fig. 1), but if the mobility of the part where it is to be placed is great there should be a free segment of wire 1 to 2 cm. long left between the electrode and the end of the tubing.

The completed electrodes are sterilized before operation in an autoclave without damage and can be promptly used thereafter.

Mode of implanting the electrodes. In applying the electrodes the nerves must be handled with the utmost care lest they be permanently damaged. In all cases ether anesthesia is used.

Cervical sympathetic or vagus. The carotid sheath is exposed through a midline ventral incision and the vagus and sympathetic trunks isolated from neighboring structures about 1 cm. below the thyroid cartilage. By very careful dissection the two nerves are separated from each other for a total distance of about 2 cm. A large cutting needle, to which the enameled wire with its rubber covering has been threaded, is now directed dorsally, from the site of the dissection of the carotid sheath, to emerge at the back of the neck as near as possible to the vertebral column and the occiput. This insures as little motion of the outgoing wire as is possible. After the electrode is properly placed, the nerve is slipped gently into it and with a pair of forceps the curved silver wire (fig. 1, *S*) is bent in a sharper curve so that the open slit in the rubber is closed. The rubber membrane is now folded about the electrode and both are fixed in place by suturing across them the cut fascial layers of the carotid sheath. No further attachment is necessary and the muscle and skin incisions can be closed.

Vagus in the thorax. The thorax is entered in the third interspace and the ribs retracted by a mechanical spreader. After the lungs are drawn downward from the apex by means of a moist, warm, silk cloth, the vagus nerve comes into view. In the right side it is easily dissected from the trachea and slipped into the lumen of the electrode. On the left the recurrent branch must first be separated from the main trunk, where the two run parallel above the aortic arch, and then the trunk can be put in place. The cut pleural surfaces are sutured over the electrode to protect the lung. In either case the enameled wire is carried in the large needle through the second intercostal space to appear in the back, medial to the superior angle of the scapula.

For convenience the splanchnic and lumbar applications may be made at the same time and through the same opening. In both a midline abdominal incision is made and during operation the viscera are held outside the abdominal cavity in a warm, moist towel.

Splanchnic nerves. The splanchnic trunks, major and minor, are isolated just as they emerge from beneath the *crus* of the diaphragm, and, as before, the needle carrying the insulated wires is passed dorsally parallel to the trunks to come out in the middle of the back. After the nerves have been slipped in place, the slit closed, and the membrane folded about the electrode, the parietal peritoneal edges are sutured together over it and, with the viscera replaced, the abdomen is closed.

Lumbar sympathetic. Here there are two trunks running parallel, both of which are exposed and isolated at about the level of the sixth lumbar vertebra. In this case the needle is directed between the vertebral column and the medial border of one psoas muscle and goes out through the back. The two nerves are slipped into the electrode which is then closed and held in place by suturing over it the edges of the psoas muscles.

At the region of exit of the wires from the back the hair is clipped, and the skin kept clean by careful washing with alcohol to avoid the entrance of any pyogenic bacteria. The electrodes may be left in place for weeks without any local or general disturbance. The animals manifested no discomfort because of their presence. Post-mortem examination shows the electrode well walled off by a proliferation of connective tissue about it. The enclosed nerve is embedded in a fibrinous exudate which fills the lumen of the electrode. Aside from rare skin infection there is no reaction around the wire leading outward.

RESULTS. An interrupted current from a Harvard induction coil, minimal for producing motor effects, was used for stimulating the nerves. Any two wires or one wire and an indifferent electrode, applied to an area of skin moistened with salt solution, may serve as connections. The nerves must not be stimulated for the first 2 or 3 days after the operation while the tissues surrounding the electrode are still sensitive from being traumatized.

If they are stimulated during this period of healing, and discomfort results the animal may be unfavorably conditioned.

When a nerve was stimulated through contacts at two electrodes in the animal's body, the usual position of the secondary coil was at 9 cm. with a 60 to 70 degree angle. The indifferent electrode on the skin necessitates a horizontal position between 8 and 8.5 cm. With the tongue as an indicator the former stimulus was just perceptible, but in the latter position it was slightly painful. The primary coil carried six volts.

Cervical sympathetic. When a slowly intensified stimulus is applied to this nerve, gradual dilatation of the pupil, retraction of the nictitating membrane, and widening of the palpebral fissure are observed, without any signs of distress on the part of the animal (see fig. 2, A and B). This evidence that pain fibers are absent from the cervical sympathetic strands is quite in accord with that of Langley, Davis and Pollock, and others, cited above.

Vagus. Stimulation of the vagus in the neck causes such vigorous reflex coughing that it is impossible to determine other vagal effects. Stimulation of the nerve through electrodes placed below the recurrent branch, however, is not in the least disturbing. The heart rate can be reduced 60 to 100 beats per minute (see fig. 3) as the animal lies on the table, quite unperturbed. This evidence supports the conclusions of Lennander and Foerster already mentioned.

Splanchnics. On gradually increasing the stimulus to these nerves, there is definite evidence of irritation, for the animal promptly curls up on its side or makes abnormal movements. If the stimulus remains constant at a slight intensity, however, there is apparently an adaptation to it (cf. Adrian, 1928), and the animal lies quietly purring on the table while the current is running. A subsequent slight increase again brings about evidence of irritation. When a moderately strong stimulus is applied, for a brief instant, there is activity of the animal that indicates disagreeable sensation.

Lumbar sympathetic. The motor effects of impulses passing down the lumbar sympathetic nerves are shown in figure 2, C and D. With an adequate stimulus the tail hairs flare up until they give the appearance of a bottle brush, without evidence that the animal is in any sense aware of the change. This observation accords with the recent evidence reported by Moore and Singleton (loc. cit.) that pain from the hind limbs is not mediated by way of lower abdominal sympathetic strands. As other observations in a number of animals showed, when the electrodes are placed at the level of the third lumbar vertebra or above, the stimulus is likely to be irritating, just as when the splanchnics are involved.

The reactions of animals when the splanchnic nerves or upper lumbar strands were stimulated stood out in marked contrast to the animal's seren-

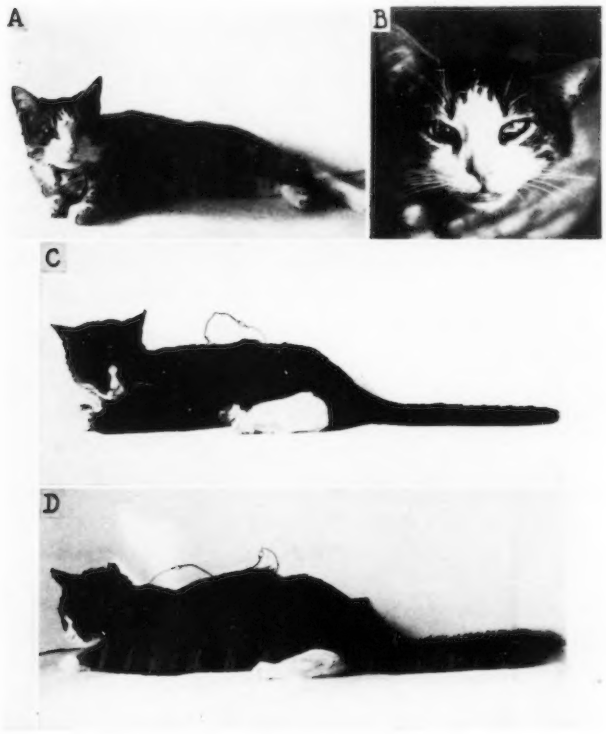

Fig. 2. Photographs demonstrating absence of excitement in unanesthetized animals while intact sympathetic nerves were being stimulated.

A. Cat, with electrode implanted in its right cervical sympathetic trunk (wires are behind cat's head, but can be seen above hind legs); in bright light the pupil is enlarged during stimulation.

B. Close view of same cat showing dilatation of the pupil, retraction of nictitating membrane, and widening of the palpebral fissure during stimulation. The head was held by hand because of photophobia from direct frontal illumination.

C. Cat with electrodes on lower abdominal sympathetic chains, before stimulation.

D. Same, during stimulation, showing pilomotor response.

Fig. 3. Record showing inhibition of the heart rate on stimulation of the left vagus below the recurrent branch in an unanesthetized animal, undisturbed by the procedure. Time, 5 seconds.

ity when an equal stimulus was applied to the cervical or lower lumbar sympathetic strands or to the vagus nerve below the recurrent branch.

SUMMARY

1. A method is described for stimulating autonomic nerves in the unanesthetized animal by means of implanted electrodes (see fig. 1).

2. The absence of pain fibers in the cervical and lower lumbar sympathetic chains and in the vagus nerve below the recurrent branch is shown by the absence of signs of disturbance when stimuli are sufficient to produce motor effects (see fig. 2, A-D and fig. 3).

3. The splanchnic nerves and upper lumbar sympathetic chains (1-3L) contain pain fibers, which show evidence of becoming adapted to prolonged weak stimulation.

4. The vagus nerve can be stimulated below the recurrent branch and the heart slowed 60 to 100 beats per minute, without disturbance of the animal (see fig. 3).

I wish to express my thanks to Dr. W. B. Cannon who pointed out the desirability of devising a method for making these observations, and to Dr. A. Rosenblueth for his help in securing the photographs.

REFERENCES

ABRASHANOW, A. A. 1927. Arch. f. Klin. Chir., cxlvi, 420,
ADRIAN, E. D. 1928. The basis of sensation. New York.
CLEVELAND, D. A. 1932. Journ. Comp. Neurol., liv, 35.
DAVIS, L. AND L. J. POLLOCK. 1932. Arch. Neurol. and Psych., xxvii, 282.
FOERSTER, O. 1927. Die Zeitungsbahnen des Schmerzgefühls. Berlin, p. 34.
FOERSTER, O., H. ALTENBERGER AND F. W. KROLL. 1929. Zeitschr. f. Neurol. u. Psych., cxxi, 139.
FRIEDRICH, H. 1924. Klin. Wochenschr., iii, 2035.
KAPPIS, M. 1920. Med. Klin., xvi, 409.
LANGLEY, J. N. 1900. Journ. Physiol., xxv, 244.
LEBEDENKO, W. AND S. BRJUSSOWA. 1930. Zeitschr. f. d. ges. exp. Med., lxxi, 198.
LENNANDER, K. G. 1906. Mitt. a. d. Grenzgeb. d. Med. u. Chir., xvi, 24.
MOORE, R. M. AND A. O. SINGLETON. 1933. This Journal, civ, 267.
RANSON, S. W. AND P. R. BILLINGSLEY. 1918. Journ. Comp. Neurol., xxix, 355.
SCHILF, E. AND R. STAHL. 1925. Klin. Wochenschr., iv, 1390.
SHAWE, R. C. 1922. Brit. Journ. Surg., ix, 450.
SPIEGEL, E. A. 1927. Handbuch der normalen und pathologischen Physiologie. Berlin, 1074.

SOME CIRCULATORY PHENOMENA DISCLOSED BY ERGOTOXINE

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From the Laboratories of Physiology in the Harvard Medical School

Received for publication May 8, 1933

That there are sympathetic vasodilators is now generally recognized. Whether a given structure is exclusively innervated by either sympathetic constrictors or dilators or whether it may receive both kinds of fibers is an important question. In a recent paper Cannon and Rosenblueth (1933) have reported that the splanchnic nerves contain a negligible component of dilator fibers when compared with the sympathetic supply to the limbs. It was deemed desirable to study further the distribution of sympathetic dilators, employing ergotoxine (Dale, 1906) to paralyze the constrictors. The use of ergotoxine not only gave an answer to the original question but revealed several other interesting circulatory phenomena, which are reported in this paper.

METHOD. Cats were used. In some cases dial anesthesia was employed; in others ether was administered until destruction or transection of the central nervous system rendered the animal insensitive to pain. The brain was sectioned by means of Sherrington's decerebrator, with the carotids ligated and the vertebrals compressed in the usual manner. The behavior of the preparations and postmortem examinations defined the level of these sections. Decapitation was effected by Sherrington's (1929) technique.

The lungs were hyperventilated in all cases after ergotoxine (Dale, 1906) through a tracheal cannula. All injections were given intravenously. The blood pressure was recorded by a mercury manometer from a femoral or, more commonly, a carotid artery. The ergot preparation employed was ergotoxine ethanesulphonate (Burroughs, Wellcome).

RESULTS. A. *Distribution of sympathetic vasodilators.* The two vascular territories contrasted were the splanchnic area and regions containing much skeletal muscle. After the injection of a moderate dose of ergotoxine, i.e., a dose insufficient to convert the action of adrenin into a pure fall of blood pressure (Dale, 1906; Cannon and Rosenblueth, loc. cit.), the fall elicited by adrenin from the whole vascular system was compared with the response from the thoracic and abdominal areas, isolated by ligation of the vessels to the head and limbs. Although the fall was usually less marked

in the reduced preparation, the exclusion of skeletal muscle was far from complete, since the thoracic and abdominal walls still received their blood supply.

The exclusion of the abdominal viscera from the circulation, however, is readily performed. We clamped the aorta at the diaphragm, rather than ligating the abdominal arteries, in order to avoid a manipulation of the viscera damaging to the blood pressure. Figure 1A shows the preponderant rise of blood pressure in response to 0.5 cc. adrenalin, 1:100,000, in a spinal preparation with the brain pithed, after the injection of ergotoxine (3 mgm. per kgm.). Three minutes later the aorta was clamped at the diaphragm, the blood pressure rose from 136 to 196 mm. Hg. Figure 1B

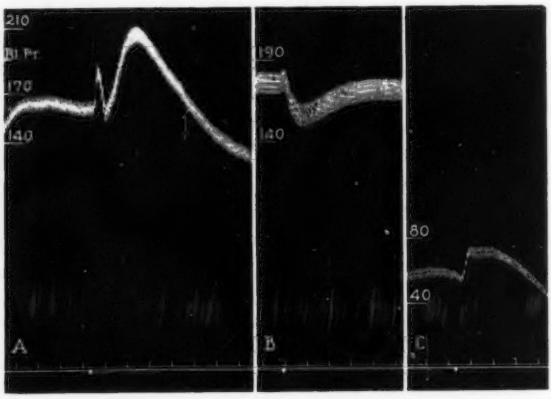

Fig. 1. Spinal animal. Ergotoxine, 3 mgm. per kgm. Time in this and other records 30 seconds.

A. Adrenalin, 1:100,000, 0.5 cc.

B. After aorta clamped at diaphragm. Adrenalin, same dose as in A.

C. After clamp on aorta released. Adrenalin, same dose as in A and B.

shows the effects of the same dose of adrenalin injected 12 minutes later—there was a pure fall. This fall is more significant because the dose of adrenalin was relatively increased by the reduced circulation. The clamp on the aorta was then released; the blood pressure fell sharply, and adrenin thereupon evoked a pure rise of blood pressure, as shown in figure 1C.

Since the blood pressure rises considerably on clamping the aorta, this circumstance might be supposed to favor the fall with adrenalin. It might likewise be argued that the low blood pressure after the release of the clamp on the aorta is the cause of the rise which is then evoked by adrenalin. That such is not the case, however, is shown by the records reproduced in figure 2. At A, 0.2 cc. adrenalin, 1:100,000, was injected into an animal

with the aorta clamped at the diaphragm and with no ergotoxine. Although the blood pressure was very high, 150 mm. Hg, and although the dose of adrenalin injected was smaller than that employed in the experiment of figure 1, there was a rise of 24 mm. Hg. Figure 2B illustrates the rise due to adrenalin in a decerebrate preparation after ergotoxine (3.5 mgm. per kgm.); the very low blood pressure of 54 mm. Hg will be explained later. The aorta was then clamped. The blood pressure rose to 160 mm. Hg. Adrenalin now produced the fall shown in figure 2C. Eighty minutes later, while the clamp on the aorta was still in place and the blood pressure was 58. mm. Hg, adrenalin still produced the fall shown in figure 2D. Figure 2E illustrates a definite fall after ergotoxine in another preparation, even though the blood pressure was only 58 mm. Hg.

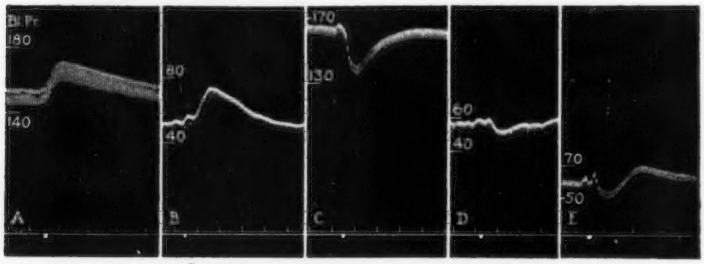

Fig. 2. A. Dial. Vagi cut. Aorta clamped at diaphragm. Adrenalin, 1:100,000, 0.2 cc.

B. Decerebrate. Vagi cut. Ergotoxine, 4 mgm. per kgm. Curare. At signal, adrenalin, 1:100,000, 0.5 cc.

C. Same animal as in B, after aorta clamped at diaphragm. Adrenalin, same dose as in B.

D. Same animal as in B and C. Eighty minutes after C, aorta still clamped. Adrenalin, same dose as in B and C.

E. Decorticate. Ergotoxine, 5 mgm. per kgm. Curare. At signal, adrenalin, 1:100,000, 1 cc.

B. *The influence of the medulla on the changes of blood pressure produced by ergotoxine.* Dale (1906) reported a large rise of blood pressure on injecting ergot preparations. This rise was best sustained by hyperventilating the lungs. Cannon and Rosenblueth (1933) confirmed these observations. Dale worked with animals decapitated or under ether or A. C. E. anesthesia and curare; Cannon and Rosenblueth used some spinal animals and some under dial anesthesia. In the present experiments the use of decorticate or decerebrate preparations to investigate certain reflexes revealed the surprising fact that in these cases the blood pressure invariably dropped considerably on injecting ergotoxine, and remained thereafter at a low level, notwithstanding hyperventilation of the lungs.

Sections at different levels of the central nervous system before the administration of the drug show that the fall invariably occurs if the medulla maintains its normal connections with the spinal cord, whatever higher centers are destroyed and although the vagi are cut. Figure 3 illustrates the marked contrast conditioned by the presence (A) or absence (B) of the medulla in the animals. In a normal animal, injected with ergotoxine after an acute, low (D. 10) spinal section, and after section of the vagi (the blood pressure being taken from the femoral artery), the blood pressure fell as in figure 3A.

The low blood pressure, when the medulla is intact, occurs even if curare is injected before the ergotoxine. If, however, curare is given after

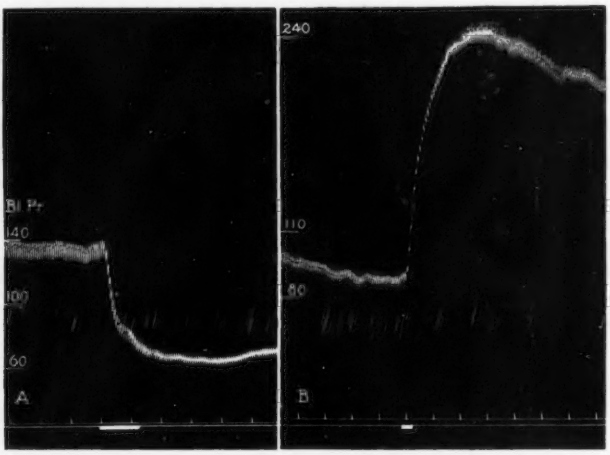

Fig. 3. A. Decorticate. Curare. Effects of ergotoxine.
B. Spinal. Curare. Effects of ergotoxine.

ergotoxine has produced the marked fall, the blood pressure usually rises to a slightly but definitely higher level, as the muscular activity of the animal (see below) progressively disappears. Figure 4A illustrates this effect of curare. The same effect is obtained by giving curare to a spinal animal in which ergotoxine has previously produced a high blood pressure (fig. 4B). In a preparation such as that exemplified in figure 3A an increase of blood pressure may likewise in some instances be obtained by a section separating the medulla from the spine. This rise, however, is more inconstant than that obtained by curare. The significance of all these data will be considered in the discussion.

C. *Vasomotor and cardiac reflexes.* It has been repeatedly shown that

even small doses of different products derived from ergot abolish most vasomotor reflexes (see Wright, 1930, for references). This observation was confirmed in the present experiments, but, even with the large doses of ergotoxine used (up to 8 mgm. per kgm.), a fall of blood pressure may be

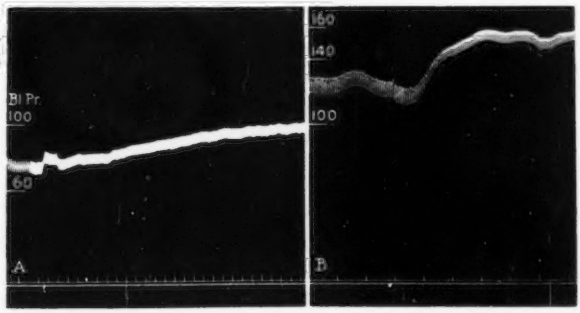

Fig. 4. A. Decerebrate. Ergotoxine, 4 mgm. per kgm. At signal, curare.
B. Spinal. Ergotoxine, 3.5 mgm. per kgm. At signal, curare.

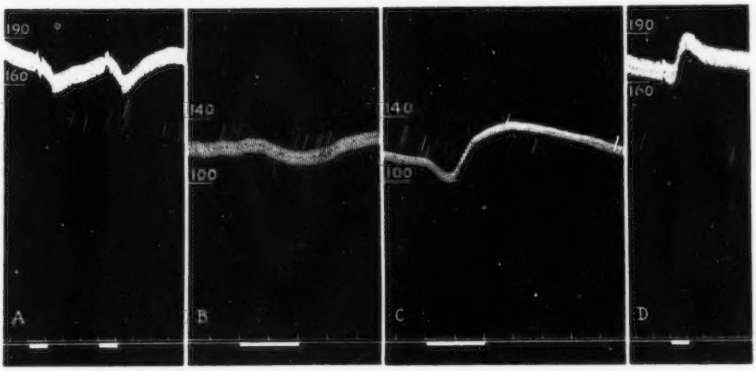

Fig. 5. A. Dial. Vagi cut. Ergotoxine, 4 mgm. per kgm. Central stimulation of the sciatic.
B. Spinal. Ergotoxine, 4 mgm. kgm. Curare. Central stimulation of the sciatic.
C. Same animal as in B. Peripheral stimulation of the sciatic.
D. Same animal as in A. Peripheral stimulation of the cervical sympathetic.

obtained from central stimulation of the sciatic (fig. 5A), an effect which persists, though lessened, if curare is administered to prevent any reflex muscular contraction (fig. 5B).

Care must be taken in stimulating the sciatic centrally to prevent spread

to the portion of the nerve connected peripherally, for if this is stimulated the fall due to the dilator fibers in the nerve, succeeded by the rise due to sympathin E (Cannon and Rosenblueth, loc. cit.), appears consistently, as shown in figure 5C. A similar misleading response occurs if, when the vagus is stimulated, the current spreads to the neighboring peripheral cervical sympathetic (fig. 5D).

It is interesting that the cardiac reflexes persist even after large doses of ergotoxine. They are modified, however, but in the sense which would be expected from some known actions of ergotoxine: central stimulation of the vagus (Moore and Cannon, 1930) and increased efficacy of the direct stimulation of the same nerve (Dale, 1906). Reflex slowing of the heart by afferent stimulation of the left vagus or depressor is slighter, whereas reflex acceleration by strong, tetanizing, central stimulation of either the sciatic or the median is more marked after ergotoxine than before.

D. *Influence of ergotoxine on the heart rate.* In all cases, whether the vagi were intact or severed, the heart beat more slowly after ergotoxine. Because of the double action of ergotoxine on the vagi (see section C), the slowing is more puzzling when the vagi are cut. With severed vagi, slowing occurred as follows in typical cases: a, dial, from 156 to 124 beats per minute; b, spinal, from 160 to 104; c, decorticate, from 276 to 164. Moore and Cannon (loc. cit). did not find any significant changes in the heart rate of vagotomized animals. Their observations, however, were performed after degeneration of the severed vagi, whereas in the present instances the vagi were cut at the time of the experiments. Several explanations occur: either ergotoxine acts not only centrally but likewise on the cardiac nerve endings; or perhaps a lasting vagal denervation desensitizes a peripheral effect of ergotoxine, i.e., an effect exerted beyond the nerves; or finally the larger doses of ergotoxine used in these experiments paralyzed centrally the accelerator tone. There was not a peripheral block of accelerator impulses, for adrenin was quite effective in increasing the heart rate. No experiments were devised to test these hypotheses.

E. *Neuromuscular effects of ergotoxine and their circulatory consequences.* The capacity of ergot preparations to elicit contraction of skeletal muscles has long been known (see Dale, 1906, for references), but no special study of the phenomenon has been reported. These motor effects are of central origin, for they are abolished by section of the motor nerves or by curare, and are, therefore, to be distinguished from the direct contractile action of ergotoxine on several smooth muscle organs. Anesthesia is apt to abolish the motor effects—dial, for instance, prevents their appearance. It is worth mentioning that dial has some depressive effect on the spinal cord, since unanesthetized spinal preparations invariably are active after ergotoxine. Suitable unanesthetized animals, decorticate, decerebrate or decapitate, are therefore desirable in studying these motor phenomena.

In all these preparations, as in the normal animal, the general motor picture is the same,—a generalized tonic background, from which clonic contractions emerge at the slightest provocation. Spinal shock is readily abolished. The increased reflex excitability resembles in its degree that produced by moderate doses of strychnine. It is in marked contrast with the disappearance of the vasomotor reflexes.

This general picture, however, differs from one animal to another, dependent on where the central nervous system was transected. The differential features are merely accentuations of the usual motor pattern obtained without ergotoxine. In the animal with low spinal section, comparable to the intact animal, continuous disorderly movements appear, without any systematization of the hypertonicity. The decorticate animal passes into typical decerebrate rigidity. The decerebrate preparation shows exclusively an exaggeration of the pre-existing motor pattern. The spinal animal, finally, exhibits as a rule a predominant flexor tone, but extensor responses are easily obtained. In a deafferented spinal segment of the animal, marked extensor rigidity appears, proving that ergotoxine not only increases reflex excitability but stimulates the motoneurones directly.

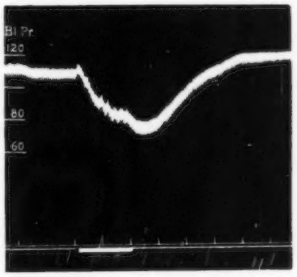

Fig. 6. Decerebrate. Ergotoxine, 4 mgm. per kgm. Effects of reflex muscular activity evoked by releasing a leg.

That these motor phenomena have a bearing on the circulatory picture is shown by the fact that struggle, i.e., sufficiently generalized muscular activity, produces a fall of blood pressure in the ergotoxinized animal (see fig. 6). It is pertinent to recall the rise of blood pressure which occurs after curare, as mentioned in section B.

Discussion. The evidence presented in section A shows that the pressor effect obtained from adrenin after a moderate dose of ergotoxine is primarily localized in the splanchnic area, while in the rest of the organism the depressor response is dominant (see fig. 1). The simplest explanation is that offered by Cannon and Rosenblueth (loc. cit.), i.e., that on blood vessels the splanchnics have primarily constrictor effects and that their dilator influence is minimal. This evidence is consistent with the theory proposed by Cannon and Rosenblueth for the transmission of sympathetic nerve impulses to smooth muscle. The theory states that the response, either contraction or relaxation, is conditioned by the pre-existing E or I substance in the responding cell. The response depends, then, on the characteristics of the muscle, not of the nerve supplying it. All that is known of nerve physiology accords with this view. That certain cells of a given vessel might respond by contraction while other neighboring cells

might relax when their respective nerves are stimulated is hardly probable. The response would in no case be the same for all the cells in the vessel if selective stimulation of one set of nerves should occur, since Cannon and Rosenblueth found that muscles with the I response (inhibitory) to sympathetic impulses do not react to sympathin E (excitatory), and vice versa. Furthermore, the selective stimulation of one set of nerves is improbable, for, as Cannon (1920) has shown, the anatomical and physiological organization of the sympathico-adrenal system insures wide distribution of the stimuli, not only in a given territory, but throughout the organism.

The distribution of both sympathetic dilators and constrictors to the same vessel or organ would then result in uncoördinated antagonistic effects, which knowledge of other biological mechanisms leads us to consider improbable. The more consistent alternative view, i.e., that a given vessel or organ responds exclusively by constriction or dilatation when acted on by the sympathetic, agrees on the other hand with the available data. The usual pressor effects of adrenin would then occur because of constriction in the splanchnic area and the skin: the depressor component, unmasked by ergotoxine, would be due to dilatation in skeletal muscle (Hoskins, Gunning and Berry, 1916; Hartman and Fraser, 1917; Hartman and Kilborn, 1918; Gruber, 1918) and other structures (e.g., the coronary vessels).

The data presented in section B require a detailed analysis. As pointed out by Dale (loc. cit.), the rise of blood pressure evoked by ergot in spinal preparations (fig. 3B) or in anesthetized animals is of peripheral and not of central origin, since it may be obtained after destruction of the whole central nervous system.

The fall (fig. 3A) which occurs when the medulla has its spinal connections is, however, decidedly a central phenomenon since it does not occur if the medulla has been destroyed before ergotoxine is injected. That it is not of cardiac origin through the vagi is proved by the record and by its appearance after section of these nerves. It is not related to respiratory phenomena, for the animals in these experiments were always given the same abundant artificial respiration immediately after the injection of ergotoxine.

Since muscular activity occasions a fall of blood pressure (fig. 6), and since ergotoxine evokes generalized muscular activity (section E), it might seem reasonable to attribute the fall of blood pressure on injecting ergotoxine to this factor. That there is a muscular component in the maintenance of a low blood pressure is shown by the rise occurring when curare is injected after ergotoxine (fig. 4). This explanation, however, is inadequate, for the blood pressure falls even if curare is injected before ergotoxine. Furthermore, non-curarized spinal preparations show, likewise, muscular activity, and the blood pressure rises.

The idea that inhibition of the vasoconstrictor center in the medulla might account for the low blood pressure is inadmissible, for ergotoxine paralyzes vasoconstrictor sympathetic impulses while stimulating smooth muscle in the vessels peripherally.

Apparently the only explanation for the phenomenon that is left is that ergotoxine stimulates a dilator center in the medulla. This center may or may not be connected with sympathetic dilators. That there exists a dilator center which is not connected with the sympathetic is shown by the reflex changes of blood pressure in completely sympathectomized animals (Freeman and Rosenblueth, 1931; Bacq, Brouha and Heymans, 1932). Destruction of the medulla after the response to ergotoxine is obtained produces only inconstantly a slight rise of blood pressure (section B). This does not invalidate, however, the explanation offered above, but is quite in keeping with the evidence that ergotoxine appears to act as it enters the cell, since, when successive doses are administered, only the first one raises the blood pressure, the succeeding doses producing if anything a fall (Dale, 1906).

The fall of blood pressure on reflex struggle (fig. 6) in ergotoxinized animals seems to us to be strictly comparable with that observed by Freeman and Rosenblueth (1931) in completely sympathectomized animals. Here, as there, a dilator mechanism, independent of the constrictor tone, must be invoked in order to explain the phenomenon.

SUMMARY

After a moderate dose of ergotoxine a dose of adrenin, which gives an almost pure rise of blood pressure in the whole animal (fig. 1A), produces a pure fall if the circulatory area is limited by clamping the aorta at the diaphragm (fig. 1B). This difference in effect does not depend on the original blood-pressure level (fig. 2). From this fact and other evidence previously reported, the inference is drawn that the splanchnics have primarily vasoconstrictor action and that probably sympathetic vasodilators distribute mainly to skeletal muscle. The bearing of this selective distribution on the theory of transmission of the sympathetic nerve impulses to the effectors, recently developed by Cannon and Rosenblueth (1933), is discussed.

In the spinal animal ergotoxine causes a rise of blood pressure (fig. 3B), whereas in the myelencephalic preparation ergotoxine produces a considerable fall (fig. 3A). This difference is explained by the excitation of a dilator center in the medulla by ergotoxine.

Although most vasomotor reflexes are readily abolished by ergotoxine, some persist after doses of ergotoxine sufficient to transform the blood pressure response to adrenin into a pure fall (fig. 5). The cardiac reflexes

persist, modified by the central and peripheral effects of ergotoxine on the vagi.

Some of the motor effects elicited by ergotoxine in different preparations are described. The most noticeable characteristic seems to be a marked reflex hyperexcitability of all motor centers.

Muscular activity lowers the blood pressure of ergotoxinized animals (fig. 6). Injection of curare, on the contrary, is followed by a rise (fig. 4).

The central effects of ergot appear thus to be complex: a, paralysis of the respiratory center; b, abolition of most vasomotor reflexes without abolition of cardiac reflexes; c, excitation of the vagal center; d, excitation of a dilator center in the medulla; e, increased reflex excitability of the spinal and encephalic motor centers; f, direct excitation of motoneurones.

REFERENCES

BACQ, Z. M., L. BROUHA AND C. HEYMANS. 1932. Compt. Rend. Soc. Biol., cxi, 152.
CANNON, W. B. 1920. Bodily changes in pain, hunger, fear and rage. New York.
CANNON, W. B. AND A. ROSENBLUETH. 1933. This Journal, in press.
DALE, H. H. 1906. Journ. Physiol., xxxiv, 163.
FREEMAN, N. E. AND A. ROSENBLUETH. 1931. This Journal, xcviii, 454.
GRUBER, C. M. 1918. Ibid., xlv, 302.
HARTMAN, F. A. AND L. McP. FRASER. 1917. Ibid., xliv, 353.
HARTMAN, F. A. AND L. G. KILBORN. 1918. Ibid., xlv, 111.
HOSKINS, R. G., R. E. L. GUNNING AND E. L. BERRY. 1916. Ibid., xli, 513.
MOORE, R. M. AND W. B. CANNON. 1930. Ibid., xciv, 201.
SHERRINGTON, C. S. 1929. Mammalian physiology. Oxford.
WRIGHT, S. 1930. Journ. Physiol., lxix, 331.

STUDIES IN RACIAL METABOLISM

Basal Metabolism of the Araucanian Mapuches

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Received for publication May 22, 1933

The relation of race to basal metabolic rate constitutes a physiological as well as an anthropological problem of great importance. The present observations on the Mapuche Indians of southern Chile were made at the Franciscan missions of Puerto Saavedra and Puerto Dominguez.

Historical. Eijkmann (1896) studied the basal metabolism of natives and settlers at Batavia, Dutch East Indies, and concluded that living in the tropics does not affect the basal metabolic rate. On the other hand, Ozorio d'Almeida (1920) noted in the white inhabitants of Brazil that the average rate was −24 per cent using the standards of Aub-DuBois; moreover, white Cubans studied in Havana by Montoro (1922) were found to have a rate of −15 per cent.

DuBois (1927, p. 159) in his summary shows that the average basal metabolic rates observed in the colored races are usually below the normal standards of white people. Malayans were −6.3 per cent; Japanese males −5.5 per cent, and females −7.3 per cent; Orientals observed in Boston were −10.2 per cent; Bengales, −9 per cent and Philippinos −8.3 per cent. In a recent paper F. G. Benedict (1932) gives the results of new researches:

	per cent
Tamils of Madrás	−17.4
Mayalis	−16.1
South Australian males	−14.0
South Australian females	−16.0
Chinese females (born in Boston)	−9.2

From these results it can be assumed that the basal metabolism depends more on racial than on climatological factors.

The several expeditions to Yucatan for the study of the Mayas, organized by Harvard University and the Carnegie Institution of Washington, are of special interest in relation to observations on the Mapuches. Williams and Benedict (1928) observed in 32 Mayas an average metabolism 5.3 above the normal for white Americans. In a second expedition Shattuck

and Benedict (1931) confirmed these results. The higher metabolic rate occurred despite a slow pulse. As this was the first instance of dissociation between pulse rate and basal metabolism, Steggerda and Benedict (1932) have recently carried on a third set of observations on 30 Mayas recording a deviation of 8.4 per cent above the standards for white individuals with extreme values of −4.5 per cent to +26.1 per cent. The average pulse rate was 52 with extremes of 41 and 80. These observations are of special interest in relation to the present work since they are the only observations on record concerning a native American race.

Preparation and selection of subjects. Our observations were made under optimal experimental conditions using carefully regulated laboratory apparatus described below. The rooms were always well aerated, isolated and constructed of wood. In all cases the observations were carried on at temperatures between 18° and 20°C. The night before the determinations the subjects were lodged at the mission; in this way we made certain that they had the proper rest and were in the post-absorptive state. They were fed a meal poor in protein and then no more food was given till after the determinations were completed.

In order to prevent any increase in metabolism from emotional strain only those subjects were accepted who, moved by small gifts, offered themselves for the observations. To this end we were greatly assisted by the mission Fathers. The majority of subjects, on the day preceding the determinations, were allowed to observe the process in another person in order to remove any remaining fear they might have entertained. Our rooms were provided with four beds, two for the subjects on whom determinations were to be carried out, and the other two for those who were to follow the next day. Observations were made in triplicate on 40 subjects and the other 5 in duplicate.

In 22 cases the determinations were repeated on two different days; no significant differences were noted between the observations of the first and second day. The oxygen intake in 8 cases was slightly higher on the first day; in 14 on the second, the average of the determinations made on the first day being 253 and of the second 255 cc. per minute.

A portable Benedict-Roth apparatus was used and a Popular Krogh set as well. The reliability of the Benedict-Roth apparatus had been checked by determining the oxygen required for burning alcohol (Benedict, 1925; Pi-Suñer, 1931). The other set was tested by frequent determinations of our own basal metabolisms and the results obtained always agreed with those observed with the Benedict apparatus.

The race observed. The Mapuches are the most numerous natives of Chile. This race has had very little intermixture in the province in which the work was done. Today the Mapuches occupy the territory between the Bio-Bio and Calle-Calle rivers, from the Andes to the Pacific Ocean,

approximately between 37° and 40°S. Living conditions among the Mapuches are very poor; they live in "rucas," cabins of straw. Due to economic and social conditions they eat very little meat and their diet is poor in protein. The average outdoor temperature when these observations

TABLE 1

31 males

NAME	AGE	HEIGHT	WEIGHT	PULSE PER MINUTE	CALORIES	DEVIATION A.-D. B.	CALORIES PER 24 HOURS	DEVIATION H.-B.	O_2 PER MINUTE
		meters	*kgm.*		*m²/hour*	*per cent*		*per cent*	*cc.*
1. P. L.	27	1.61	77	60 62	34.4	−13	1,505	−13	226
2. P. L.	21	1.51	52	64 62	42.3	+8	1,495	+7	213
3. J. C.	20	1.57	59.5	64 64	46.7	+14	1,795	+15	259
4. J. T.	20	1.63	65	70 72	55.3	+34.4	2,172	+32.3	311
5. B. C.	35	1.65	78	58 62	38.2	−3.1	1,707	−1.3	226
6. J. D.	25	1.69	76	68 74	42	+2.5	1,885	+5.2	273
7. C. C.	38	1.70	76.5	64 60	45	+14	2,021	+18	272
8. M. P.	19	1.56	60	60 56	41.1	+7	1,651	+7	240
9. D. R.	21	1.65	70	46 49	43.6	+10	1,854	+8	250
10. S. P.	20	1.69	66	66 64	41.6	+4	1,729	+3	248
11. T. C.	28	1.62	63	65 62	42.6	+8	1,717	+9	247
12. A. R.	20	1.62	57	76 72	42.8	+8.3	1,643	+7.7	252
13. S. D.	21	1.66	63	60 62	40.6	+2.8	1,763	+8.7	256
14. F. M.	23	1.65	58.5	56 60	46.3	+17	1,823	+17	262
15. J. V.	23	1.57	57	46 44	41	+3.7	1,548	+4.5	206
16. T. D.	20	1.61	55.5	62 64	47.6	+19	1,834	+22	261
17. C. Z.	33	1.66	83	60 58	43.1	+9	1,975	+9	284
18. P. L.	20	1.66	70	60 64	45.2	+10	1,963	+13	248
19. F. B.	35	1.63	72	68 72	38.1	−8.5	1,628	−0.5	232
20. J. H.	40	1.64	68	54 52	40	+6	1,580	+2	236
21. A. M.	34	1.69	63	44 42	46.4	+17	1,912	+23	236
22. A. C.	20	1.58	68	62 58	41.5	+5	1,698	+4	241
23. A. C.	20	1.68	66	64 64	40	+4.2	1,753	+3.7	250
24. M. H.	44	1.55	65	64 62	49.8	+29	1,969	+36	280
25. F. C.	35	1.665	87	50 48	49.4	+25	2,330	+25	332
26. J. J.	30	1.635	64.5	76 72	41.5	+5	1,704	+8	245
27. F. H.	31	1.66	62	50 56	52	+31	2,121	+30	305
28. F. L.	20	1.67	64	64 60	37.7	+6	1,559	+6	222
29. C. C.	20	1.57	58	60 60	42.3	+5	1,479	+3	213
30. A. C.	26	1.68	71	52 52	44	+11	1,908	+11	273
31. D. H.	21	1.65	75	68 64	48	+10	1,920	+7.7	275
Average				60		+9.3		+9.8	

were made, in September 1932, (the beginning of spring in Chile) was 9.3°C. The minimum in the three weeks of our work was 0.4° and often at 6 a.m. the temperature ranged from 5° to 10°C.

Data. The data obtained are summarized in tables 1 and 2.

In both tables the deviations for calories/square meter/hour are calculated in accordance with the standard of values of Aub-DuBois, and the deviations for calories/day with the normal values of Harris and Benedict (1919).

Nutrition and racial type of the Mapuches. It is necessary to inquire whether the observed differences are due to abnormalities in the nutrition of the Mapuches that might have given rise to abnormal proportions of height and weight. The Pirquet index may be used as a standard. Normally the value of the formula:

$$\sqrt[3]{\frac{10 \times \text{weight in gr.}}{\text{sitting height in cm.}}}$$

TABLE 2

14 females

NAME	AGE	HEIGHT	WEIGHT	PULSE PER MINUTE	CALORIES	DEVIATION, A.-D. B.	CALORIES PER 24 HOURS	DEVIATION, H.-B.	O_2 PER MINUTE
		meters	*kgm.*		*m²/hour*	*per cent*		*per cent*	*cc.*
1. L. C.	29	1.52	65	52 50	43	+23	1,771	+23	257
2. N. L.	21	1.495	65	62 62	51	+37	1,971	+39	277
3. E. N.	25	1.55	56	84 82	44.5	+20.9	1,645	+20.9	233
4. M. J.	20	1.53	77	62 66	37.4	+1.1	1,572	−0.6	222
5. M. L.	18	1.585	73.5	60 58	44.6	+17	1,886	+20	268
6. J. B.	21	1.49	77	74 72	45.4	+23	1,874	+19	267
7. A. B.	23	1.52	83	72 74	43.4	+17	1,665	+16	237
8. C. C.	21	1.60	72	66 64	40.6	+9.6	1,714	+11	246
9. V. C.	25	1.53	58	76 70	44.2	+19	1,636	+17	233
10. I. C.	37	1.515	61.5	74 78	42.5	+16	1,612	+17	231
11. J. P.	45	1.53	73	68 60	31	−14	1,269	−11	181
12. J. M.	22	1.525	63	76 80	42.3	+14	1,627	+13	232
13. F. L.	35	1.55	53	64 64	42.4	+13	1,527	+19	219
14. T. X.	24	1.51	59	62 68	40.4	+9	1,595	+8	213
Average				67		+14.4		+14.8	

should be equal to 100 or very close to this figure. A lower value indicates emaciation; a higher, obesity. In the white race the sitting height is equal to one-half the full height plus 5 cm. Unfortunately no data could be collected in order to calculate these indices on all the subjects. However, in 26 men the pelidisi ranged from 94 to a maximum of 104; the average being 98.2. The difference between one-half the height and the sitting height averaged 6.2 cm. with values between 2.5 and 10.5 cm. Therefore, according to these values the nutritional state of the Mapuches studied is practically normal and the relation between full height and sitting height is not far from that of the white race.

DISCUSSION. The analysis of the data collected in the preceding tables indicates that among the Mapuches the basal metabolic rate is higher than among the races studied so far. Our values, +9.3 per cent and +9.8 per cent for the male sex, are slightly higher than those observed by Steggerda and Benedict (1932), +8.4 per cent among the Mayas of Yucatan. For females our values are still higher, +14.4 per cent and +14.8 per cent. Unfortunately the North American workers have not carried on any studies of subjects of the latter sex, so no comparison could be made. These results are particularly interesting in view of the fact that the diet of these subjects was poor in protein. A dissociation between pulse and basal metabolism was observed in the Mayas. Steggerda and Benedict (1932) give an average rate of 52 for the Mayas. In male subjects we found an average rate of 60 per minute and in individual cases the pulse was as low as 46 per minute. We can therefore state that the Mapuches, like the Mayas, *present the phenomenon of dissociation between pulse and basal metabolism, though to a lesser degree.*

In the case of females the dissociation is less apparent. These subjects had an average pulse of 67 and a basal metabolism of 14.4 per cent above the standards of Aub-DuBois or 14.8 per cent above those of Harris and Benedict. Yet, in comparing the values for height and weight a tendency toward obesity was observed. It is well known that non-thyroidic obesity causes cardiac acceleration. Certainly in a group of European or North American white women of the same age, size and weight, with the same basal metabolism an average pulse rate of more than 67 would be expected. It would be interesting to know whether this also occurs among the female Mayas.

Throughout this work the methods of Steggerda and Benedict (1932) have been used because their observations among the Mayas and ours on the Mapuches are the only ones so far published on the aboriginal American races. It is interesting to observe that the agglutination studies of blood grouping can be correlated with those obtained in the study of basal metabolism.

	O	A	B	AB
Mayas	76.6	16.7	5.3	1.4
Mapuches	75.0	17.8	6.2	1.0

Compare these averages with the normal in white Americans:

45.0	42.0	10.0	3.0

or of the Europeans of Latin countries:

42.0	43.6	10.5	3.9

Here again we observe that in blood groupings the Mapuches present the same phenomenon as the Mayas but to a lesser degree.

Thus, the only two American races so far studied present physiological values very similar and yet completely different from those observed for other races though the phenomena are more marked in the Mayas of Yucatan than in the Mapuches of southern Chile.

SUMMARY

The basal metabolic rates of 45 Mapuches, 31 men and 14 women, have been determined with the closed circuit type apparatus (Benedict-Roth and Popular Krogh). Associated with the high values of basal metabolism was a pulse rate slower than the normal for the white race. The average values are 9.8 per cent above Harris-Benedict standards for men and 14.8 per cent for women. The average pulse rate is 60 in men and 67 in women. Our results are similar to the observations on the Mayas of Yucatan, and are different from those on non-aboriginal American races.

I should like to take this opportunity of expressing my gratitude to Dr. F. G. Benedict for his helpful advice in the preparation of the expedition and of this paper; to Drs. Ignacio Matte, Eduardo Vinyal and Guillermo Reyes for their technical collaboration in making the observations; to Mr. Arrillaga-Torrens, student in the Yale University School of Medicine, for the English translation of the paper, and to Prof. H. E. Himwich for reading and criticising the manuscript before publication.

REFERENCES

BENEDICT, F. G. 1925. Boston Med. Surg. Journ., cxciii, 583.
1932. Amer. Journ. Physical Anthrop., xvi, 643.
DUBOIS, E. F. 1927. Basal metabolism in health and in disease. Lea & Febiger. Philadelphia. 2nd ed.
EIJKMANN, J. K. 1896 Pflüger's Arch., lxiv, 57.
HARRIS, J. A. AND F. G. BENEDICT. 1919. A biometric study of basal metabolism in man Carnegie Inst. Washington, Publication no. 279.
MONTORO. 1921–1922. San. Mil. Habana, i, 255 (quoted by DUBOIS, 1927).
OZORIO D'ALMEIDA, A. 1920. Journ. Physiol. et Pathol. gén., lviii, 712.
PI-SUÑER, J. 1931. Compt. rend. Soc. Biol., cvi, 1013.
SHATTUCK, G. C. AND F. G. BENEDICT. 1931. This Journal, xcvi, 518.
STEGGERDA, M. AND F. G. BENEDICT. 1932. This Journal, c, 274.
WILLIAMS, G. D. AND F. G. BENEDICT. 1928. This Journal, lxxxv, 634.

STUDIES ON THE FATE OF EPHEDRINE IN THE DOG

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Received for publication May 22, 1933

In previous reports from this laboratory it has been shown that the liver is much more effective in destroying or inactivating certain alkaloids than other tissues such as the heart, lungs, and voluntary muscle. Priestley, Markowitz and Mann showed that strychnine was more readily destroyed by the liver than by skeletal muscle, and Biebl, Essex and Mann found the ability of the liver to detoxify nicotine definitely greater than that of an equivalent mass of skeletal muscle.

It was our purpose in this study to investigate the ability of various tissues to inactivate ephedrine sulphate and to determine the site of inactivation of this alkaloid in the body. It seems to be generally accepted that the prolonged action of ephedrine as compared with epinephrine results from the greater stability of ephedrine, which makes its destruction by the body more difficult. The experimental work of Nukita suggested that the liver is capable of inactivating ephedrine since he found some loss of ephedrine after perfusion with Ringer's solution through the liver of dogs, cats, rabbits, and guinea pigs. The livers of rabbits manifested the greatest destructive power. It should be noted that perfusion of the liver with Ringer's solution does not give trustworthy results. Nukita also studied the pressor effect resulting from an injection of ephedrine into the mesenteric vein as compared with injection into the leg vein. Although he found the pressor effect was greater when the drug was injected into the leg vein, the method is open to the criticism pointed out by Priestley, Markowitz and Mann that a substance injected into the leg vein does not traverse a capillary bed before reaching the general circulation as compared to an injection made at a portal nodule.

All our experiments were carried out on dogs, with heart-lung, heart-lung-limb and heart-lung-liver preparations, and also on animals following the removal of the kidneys and the abdominal viscera. Anesthesia was induced by ether or amytal (iso-amylethyl barbiturate). Ephedrine was estimated by its pressor effect when injected intravenously into small dogs (3 kgm.) under amytal anesthesia, the blood pressure being taken from

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the femoral or carotid artery. The ephedrine sulphate solution in each instance was prepared immediately before use.

RESULTS. *Incubation.* Five milligrams of ephedrine sulphate were incubated for six hours with 10 cc. of whole heparinized blood, with 10 cc. heparinized plasma, with 10 cc. washed blood cells suspended in normal saline solution and with 10 cc. normal saline solution. Each specimen was subsequently injected intravenously into a dog under amytal anesthesia. An interval of forty-five to sixty minutes was allowed between injections. Each specimen produced approximately the same effect on the blood pressure as that produced by an equal amount of ephedrine freshly made up in 10 cc. normal salt solution. Injection of 10 cc. heparinized blood alone was without appreciable effect on the blood pressure. The same results were obtained when 3.0 mgm. ephedrine was similarly treated.

Heart-lung preparation. Heart-lung preparations were made and ephedrine sulphate was added in concentration of 0.3 mgm. in each cubic centimeter of the volume of circulating blood (estimated). After three to three and a half hours' perfusion through the heart and lungs, 10 cc. of blood were taken from the reservoir and injected intravenously into dogs of standard size. This produced a comparable effect on the blood pressure as did 3.0 mgm. ephedrine sulphate dissolved in 10 cc. of normal saline solution.

Heart-lung-hind limb preparation. Perfusion of the hind limbs, by means of the heart-lung preparation, was then performed. A tube from the arterial side of the circuit was connected with the abdominal aorta with minimal loss of time and the returning blood was collected from the inferior vena cava. Following these procedures the hind limbs were completely isolated from the remainder of the body. Bleeding was controlled by suture. Ephedrine sulphate in concentration of 0.3 mgm. in each cubic centimeter of circulating blood (estimated) was then added to the arterial side of the heart-lung circuit so that it first circulated through the hind limbs. After perfusion for two to two and a half hours, 10 cc. samples were collected from the inferior vena cava of the hind limb preparation. When this was injected intravenously into dogs of standard size, the blood pressure response was in each instance practically identical with that produced by the intravenous injection of 3.0 mgm. ephedrine sulphate dissolved in 10 cc. normal saline solution. It should be stated that the hind limbs at the end of the perfusion period appeared to be in excellent physiologic condition. They did not show signs of rigor, and induction shocks caused vigorous contraction of the muscles of the limb.

Heart-lung-liver preparations. Heart-lung-liver preparations were made according to the method described by Priestley, Markowitz and Mann except that the portal vein was connected to the venous reservoir so that hydrostatic pressure of 10 to 12 inches of blood was constantly maintained. The experiment was discontinued at the end of three hours. The liver ap-

peared to be in a functioning condition at the end of the experiment. Ephedrine sulphate was added to the portal vein circuit in concentration of 0.3 mgm. in each cubic centimeter of circulating blood (estimated). After three hours' perfusion, 10 cc. samples taken from the hepatic vein and injected into dogs of standard size produced practically identical pressor response to that obtained from 3.0 mgm. ephedrine in 10 cc. normal salt solution (fig. 1).

Nephrectomized animals. Since we did not find evidence that ephedrine was destroyed or made inactive by skeletal muscle or hepatic tissue, there seemed a possibility that it might be eliminated by the kidneys. If so, the pressor effect in a nephrectomized dog might be expected to be more prolonged than in the intact animal. Bilateral nephrectomy was performed on

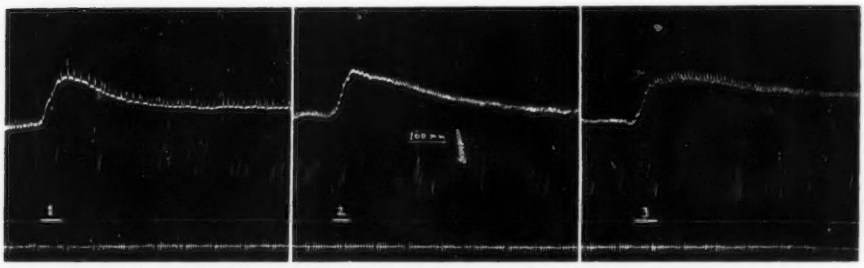

Fig. 1. The effect on the blood pressure by the intravenous injections of 10 cc. of blood which contained about 3 mgm. of ephedrine sulphate at the beginning of the experiment. The first injection (signal mark *1*) shows the pressor effect of 10 cc. of blood from the venous reservoir after perfusion through a heart-lung-liver preparation for ten minutes. The second injection (signal mark *2*) is the same except the blood had been perfused through the liver for three hours. The third injection (signal mark *3*) shows the effect of 3 mgm. of ephedrine sulphate in 10 cc. of normal saline solution. Weight of dog, 2.9 kgm.

two dogs under aseptic conditions. The following day a normal response to intravenously injected ephedrine was obtained. In one experiment the effect of the standard amount of ephedrine on blood pressure was determined before operation. The same dose given after nephrectomy produced practically an identical response.

Animals without abdominal viscera. The response to ephedrine of animals before and after removal of the abdominal viscera was not significantly different. The effect of the drug on the animal operated on was more prolonged than that on the intact animal, but injections of epinephrine were similar in this respect.

Comment. It is evident from these results that the liver perfused with defibrinated blood exerts no destructive or inactivating effect on thera-

peutic doses of ephedrine sulphate. It may be further concluded that the pressor effect of ephedrine is not altered by the blood, skeletal muscle, cardiac muscle, lungs, liver or kidneys. Elimination by the lungs or kidneys is not responsible for the gradual failure of a single dose of ephedrine to maintain its characteristic effect on the blood pressure. One is forced to conclude that ephedrine sulphate is not eliminated or destroyed by the organism within the period of time of these experiments in amounts that can be detected by the method employed. These results corroborate work of others that there is a transient refraction period to ephedrine.

SUMMARY

A series of experiments in which ephedrine sulphate was perfused through heart-lung, heart-lung-limb and heart-lung-liver preparations for from two and a half to three hours indicated that the pressor principle of this alkaloid is not destroyed or inactivated by such treatment. Contrary to what occurs in the case of strychnine and nicotine, ephedrine sulphate is not altered appreciably by passage through the liver. Ephedrine sulphate is not rapidly excreted through the lungs or kidneys. All of the tissues perfused failed to reduce the pressor effect of this drug.

REFERENCES

(1) Biebl, M., H. E. Essex and F. C. Mann. 1932. This Journal, c, 167.
(2) Chen, K. K. and W. J. Meek. 1926. Journ. Pharm. Exp. Therap., xxviii, 31.
(3) Gradinesco, A. 1927. Compt. rend. Soc. de biol., xcvi, 1027.
(4) Hildebrandt, F. and H. Mügge. 1931. Klin. Wochenschr., x, 291.
(5) Nukita, Y. 1933. (Abstract) Chem. Abstr., xxvii, 1050.
(6) Pak, C. and B. E. Reed. 1928. Chinese Journ. Physiol., ii, 435.
(7) Priestley, J. T., J. Markowitz and F. C. Mann. 1931. This Journal, xcvi, 696.

SOME DATA FROM A CORRELATED ANATOMICAL, PHYSIOLOGICAL AND BEHAVIORISTIC STUDY OF THE REPRODUCTIVE CYCLE IN THE FEMALE GUINEA PIG

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Received for publication May 16, 1933

During the past fifteen years the reproductive cycle of our domestic and laboratory mammals has been the subject of numerous investigations with the result that many obscurities of a decade ago have been more or less completely elucidated. For the most part, however, those who have studied this problem have confined themselves to rather special aspects of it. For example, the rhythmic changes which occur in the genital tract in the guinea pig have been carefully studied by Loeb (1914 and 1918) and Stockard and Papanicolaou (1917 and 1919); in the sow by Corner (1921); in the mouse by Allen (1922); and in the rat by Long and Evans (1922), but no one of these investigators supplemented his account with a really comprehensive description of the accompanying behavioristic phenomena. At the same time two other investigators, Ishii (1920) and Avery (1925), stressed the rhythmic changes in behavior but made no attempt to associate these with the internal changes described by the other group. Only Hammond (1927) in his excellent and painstaking study of the reproductive cycle in the cow has correlated observations in reproductive behavior with anatomical and histological changes in the genital tract.

To the writers, a similar study on one of the commonly used laboratory mammals seemed desirable and because observations made previously by one of us (Young, 1931) suggested that the guinea pig would be most nearly ideal, we have used this animal.

The results obtained during a preliminary experimental period in February convinced us that, for the guinea pig as for the cow, dependable data could be obtained only if the animals were observed continuously, day and night, through at least four reproductive cycles. This we have done. Working in shifts, we have not left the animals more than a half-hour at a time throughout a period of more than two and a half months. Most of the time, and especially during the night, one of us has sat beside the cages continuously. During the entire pro-oestrum, oestrus, and metoestrum, the animals were examined at half-hourly intervals and data were recorded with respect to the condition of the vaginal closure membrane,

the behavior of the females in the cages, and the assumption of the copulatory posture when touched. During the dioestrum the animals were kept in the same room and were observed constantly. Altogether 90 females were observed throughout three, four and five oestrous cycles. During this time we were able to make 17 series of vaginal smears at half-hourly intervals from the time of the first opening of the vaginal closure membrane until its closure. We were able to determine accurately, on the basis of 231 individual cycles, the length of the oestrous or reproductive cycle and its variation in length during successive cycles. We were able to ascertain the length of the oestrous period, that is, the period of receptivity, by observing our animals continuously during 343 oestrous periods. These observations also enabled us to ascertain the variation in length of successive oestrous periods in the same animal. We were able to determine the time of day when oestrus is most likely to begin and the time when most animals are likely to be found in heat simultaneously. In order to avoid misinterpretations in this latter part of the work, standard time was retained after the period of daylight saving time had begun. Lastly, we were able to determine the sequence of events which characterize oestrous behavior.

It was only after this systematic study of the oestrous behavior in normal females had been completed that additional data which would involve the sacrifice of the animals were obtained. At this time we undertook to make a study of the time of follicular development, to determine the number of follicles which develop and rupture at each oestrus, to correlate this number with the length of the oestrus, to determine the exact time of ovulation and to relate it to the other events of the cycle and period, to determine the effect of copulation upon the length of oestrus and the time of ovulation, and finally to determine the fertility of the female when mated at different times during the oestrous period.

A last part of the study was planned to be histological. Seventy-six animals were killed at twelve different stages in the established oestrous cycle and from each animal we removed and preserved both ovaries, a section of one uterine horn, a section of the vagina, one mammary gland, and the hypophysis.

The completion of the experiment along the lines we have planned and the preparation and study of the material that has accumulated will require considerably more than a year. In the meantime, many of our data are complete and since these reveal several new facts concerning the oestrous cycle in the guinea pig, they are being reported at this time.

The mean length of the reproductive cycle as we have determined it on the basis of 231 cycles is 16 days, 7 hours. This calculation is somewhat more refined but not different from the 15–17 days estimated by Stockard and Papanicolaou. From animal to animal the range was considerable.

The shortest cycle from the beginning of one oestrus to the beginning of the succeeding oestrus was 13 days. The longest cycle lasted 21 days, 12 hours. For any one animal, on the other hand, the lengths of successive cycles were much more constant. In general, the larger, older animals have longer cycles, the smaller, younger animals, shorter cycles. For all animals the coefficient of variability was 6.6 per cent. Due to the tendency of the animals to come into heat after 6 p.m., instead of during the daylight hours, the distribution curve is a bimodal curve, the maxima falling at 16 and 17 days.

The mean length of the oestrous period as we have determined it on the basis of 343 periods is 8.01 hours and does not vary appreciably from the 8 to 11 hours which was established by Ishii. Here again, the range was considerable. Two animals which appeared perfectly normal in every other respect never did come into heat even though the vaginal closure membrane ruptured regularly. With the exception of these two animals and an

TABLE 1

Number of corpora lutea and the length of the corresponding oestrous period

CORPORA LUTEA	NUMBER OF CASES	VARIATION IN LENGTH OF OESTRUS	AVERAGE LENGTH OF OESTRUS
		hours	*hours*
1	4	7.0–11.5	8.25
2	13	0.0– 9.5	6.07
3	17	0.0–14.5	7.41
4	14	5.5–10.5	7.96
5	2	6.5– 7.0	6.75
Total.....	50	0.0–14.5	7.56

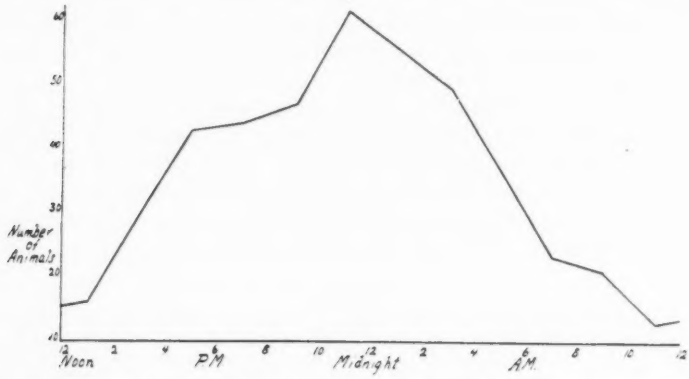
occasional "miss," the shortest period was one of an hour's duration, the longest 14.5 hours. For any one animal the lengths of successive cycles were much more constant. Once the past record was known, a fairly accurate estimation of the length of succeeding periods could be made. For all animals the coefficient of variability was 26 per cent and the distribution curve is a normal unimodal curve.

By counting the corpora lutea after the animals had been killed it was possible to determine the number of follicles which had developed prior to and during the previous oestrus. These data from 50 females are summarized in table 1.

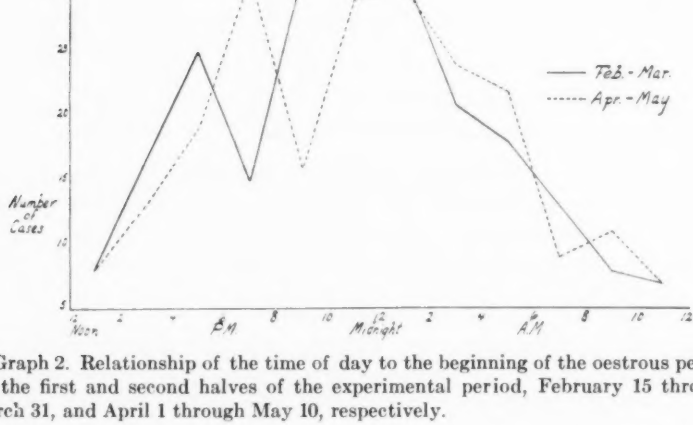
It will be seen that there is no relationship between the number of developing follicles and the length of the corresponding oestrous period. The variation in the latter is constant irrespective of the number of follicles which had matured.

One of the most interesting characteristics of the oestrous period is the

frequency with which oestrus occurs at night rather than during the daylight hours. Data from 442 observations are recorded in graph 1. It can be seen that most of the animals (66.7 per cent) come into heat between 6 p.m. and 6 a.m., the maximum of activity occurring at 11 p.m. Actually, however, all that is involved is revealed only by an examination and com-



Graph 1. Relationship of the time of day to the beginning of the oestrous period



Graph 2. Relationship of the time of day to the beginning of the oestrous period for the first and second halves of the experimental period, February 15 through March 31, and April 1 through May 10, respectively.

parison of the curves prepared from data for the two halves of the experimental period, February 15 through March 31, and April 1 through May 10 (graph 2). When this is done it is found, first of all, that animals show an especial tendency to come into heat during either of two periods in the day; late in the afternoon or early in the evening, or, if not then, later in the

evening between about ten and two. It is found, secondly, that these maxima of oestrous activity have shifted to the right as the days have become longer, that is, toward the later night and early morning hours. It is found, thirdly, that the hump to the left of the maximum in graph 1 is really a second maximum which expresses the frequency of oestrous activity in the late afternoon and early evening and which has become somewhat leveled off because of the shifting of the time of oestrous activity with the lengthening day.

Obviously, the occurrence of oestrus is subject to some nocturnal influence. What this influence can be is not understood. Nor are we able to explain why there should be two maxima of activity unless in many animals threshold changes preparatory for heat are partially built up during the last night of the dioestrum and completed early the following evening. Both problems are being investigated and will be reported upon later.

The time of ovulation in relation to oestrus has been estimated from examinations of the ovaries made immediately after the death of a series of 64 animals killed at various stages during pro-oestrum, oestrus, and metoestrum. In no one of 14 females killed an hour and a half or more after the end of oestrus had rupture of any of the mature follicles failed to occur. In only one out of six animals killed one hour after the end of oestrus had ovulation failed to occur. In ten out of sixteen animals killed at the end of oestrus or just before its end ovulation had not occurred. In five animals killed approximately one to two hours before the end of oestrus ovulation had not occurred. We conclude, therefore, that the relationship of the time of ovulation to the termination of oestrus is very definite. Irrespective of the length of the oestrous period, it would appear that ovulation occurs within an hour of the termination of oestrus.

To a certain extent, the significance of the data recorded above and the problems they suggest will be apparent. Both subjects, however, can be discussed to better advantage after there has been an opportunity to study histologically the tissues which have accumulated. We will only point out that, at the moment, the most interesting aspects of the problem are the factor or factors which operate to bring the animals into heat at night rather than, to the same extent, throughout the twenty-four hours of the day, the lack of any correlation between the number of developing follicles and the length of oestrus, and the existence of such different coefficients of variability for the length of the entire reproductive cycle, on the one hand, and the length of the oestrous period, on the other.

CONCLUSIONS

1. The mean length of the reproductive cycle in the female guinea pig is 16 days, 7 hours. Continuous observations during 231 reproductive cycles revealed that they may be as short as 13 days or as long as 21½ days.

2. The mean length of the oestrous period is 8.01 hours. Continuous observations of females during 343 oestrous periods revealed that oestrus may last only 1 hour or that it may last as long as 14.5 hours.

3. No correlation exists between the number of maturing follicles and the length of the oestrous period. Animals in which one follicle had matured remained in heat 7.0 to 11.5 hours. Animals in which four follicles had matured remained in heat 5.5 to 10.5 hours.

4. The occurrence of oestrus between 6 p.m. and 6 a.m. is twice as likely as between 6 a.m. and 6 p.m. although during a part of the year a first maximum may occur about 5 p.m. Oestrus, therefore, is distinctly a nocturnal phenomenon.

5. Irrespective of the length of the oestrous period, ovulation appears to occur within an hour of the end of oestrus.

REFERENCES

ALLEN, E. 1922. Amer. Journ. Anat., xxx, 297.
AVERY, G. T. 1925. Journ. Comp. Psychol., v, 373.
CORNER, G. W. 1921. Carnegie Inst. of Washington, Contrib. to Embryol., xiii, 117.
HAMMOND, J. 1927. The physiology of reproduction in the cow. Cambridge University Press, London.
ISHII, O. 1920. Biol. Bull., xxxviii, 237.
LOEB, L. 1914. Biol. Bull., xxvii, 1.
1918. Sci., N. S., xlviii, 273.
LONG, J. A. AND H. M. EVANS. 1922. Memoirs, Univ. California, vi.
STOCKARD, C. R. AND G. N. PAPANICOLAOU. 1917. Amer. Journ. Anat., xxii, 225.
1919. Biol. Bull., xxxvii, 222.
YOUNG, W. C. 1931. Journ. Exp. Biol., viii, 151.

THE EFFECT OF REMOVAL OF THE SYMPATHETIC CHAINS AND OF THE COELIAC GANGLIA ON GASTRIC ACIDITY

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Received for publication April 21, 1933

For over a century observers have sectioned the extrinsic nerves of the stomach in animals to study the changes in the enteric plexuses or in gastric physiology. This study describes the effect of removal of the sympathetic chains and of the coeliac ganglia on gastric secretion as measured by acidity.

Several authors have sectioned the vagus nerves, splanchnics, or both, in dogs in an attempt to control gastric acidity, and a few of the more recent results are given in table 1.

TABLE 1

Literature

AUTHOR	NERVES	SECTION	ACIDITY (FREE AND TOTAL)
Hartzell (1)	Vagi	Intrathoracic	Decreased
Vanzant (2)	Vagi (same dogs as above, two years later)	Intrathoracic	Normal
Friedenwald and Feldman (3)	Vagi separately	Both intrathoracic and subdiaphragmatic	Inconstant—returns to normal
Moll and Flint (4)	Splanchnics	Intrathoracic	Slightly increased

It is apparent that section of the vagus and splanchnic nerves has given very little permanent change in gastric acidity. These fibres, however, are preganglionic, and in the following experiments on cats, the coeliac ganglia were removed in order to section the postganglionic fibres permanently. To study permanent section of preganglionic fibres, gastric analyses were performed on 3 animals, belonging to the Physiology Department of the Harvard Medical School, which had had the entire sympathetic chains removed for a period of from $3\frac{1}{2}$ years to 2 months. Permission to perform gastric analyses on these 3 animals was kindly granted by Dr. Walter B. Cannon.

METHOD. For the 7 coeliac ganglionectomies performed by the author, intraperitoneal sodium amytal was used on the first 4 animals and ether on the last 3. A midline abdominal incision was made and the omentum, stomach and intestines were removed from the cavity and covered with warm saline sponges. The right kidney was located, and from this the right adrenal, which is attached by many short fibres to the coeliac ganglion. After going through the parietal peritoneum, the ganglia were dissected out from behind the vena cava and from around the coeliac axis until the branches to the stomach and to the left adrenal were laid bare. These fibres were tied and cut and the ganglia removed intact. The abdominal contents were then replaced and the peritoneum and muscle layers sutured with black silk. Skin clips were used, covered with a collodion dressing.

To perform gastric analyses, the cats were placed in a cat box and an ordinary stomach tube used. In the first analyses a fasting specimen was obtained when possible, and the cat given 50 cc. of 7 per cent alcohol. Stomach contents were removed after the animal had been back in its cage twenty minutes. If free hydrochloric acid was not found, the cat was then given $\frac{1}{4}$ milligram of histamine intramuscularly and gastric analysis performed again in twenty minutes. It was found that the presence of free hydrochloric acid after test meal varied greatly between animals or in the same animal from time to time, so that the test meal was abandoned. The most satisfactory method was to obtain the fasting specimen, then give the histamine and 50 cc. of water. The time period was extended to one-half hour before extraction, and fairly consistent results were obtained.

RESULTS. Each of the cats with the postganglionic fibres intact (Dr. Cannon's) had 2 gastric analyses and with histamine normal results were obtained for both free and combined acidity in all instances.

Coeliac ganglionectomy was performed on 7 cats: 4 died within a week, while the 3 which survived remained in good health, after recovery from the shock of the operation.[1] The only gross or microscopic postmortem changes were found in the gastric plexuses and in the adrenal glands; these will be described later in a report on the histological changes.

Both pre- and post-operative gastric analyses were made on the 3 animals without postganglionic fibres. On 7 occasions there was no significant change in free or total acidity at periods varying from 9 days to 5 months post-operatively.

[1] There was no bloody diarrhea nor ulceration of the gastro-intestinal tract as described by Laignel-Lavastine (5) following ablation of the solar plexus in dogs. Danisch's (6) rabbits remained well and gained in weight after this operation.

CONCLUSIONS

There was no demonstrable change in either free or combined gastric acidity following complete removal of sympathetic chains or coeliac ganglia in cats. Since the stomach may function automatically in the absence of extrinsic nerves, it is not probable that degeneration of the autonomic nervous system would lead to either hyperchlorhydria or achlorhydria.

I wish to acknowledge my indebtedness to Dr. Stanley Cobb for his advice in carrying out these experiments, and to Dr. Jacob Finesinger and Mrs. Elizabeth Linden for their assistance.

REFERENCES

(1) Hartzell, J. B. This Journal, 1929, xci, 161.
(2) Vanzant, F. R. Proc. Staff Meet. Mayo Clinic, 1932, vi, 576.
(3) Friedenwald, J. and M. Feldman. Arch. Int. Med., 1932, xlix, 234.
(4) Moll, H. and E. R. Flint. Brit. Journ. Surg., 1928, xvi, 283.
(5) Laignel-Lavastine, M. Researches sur le Plexus Solaire. Georges Steinheil, Paris, 1903.
(6) Danisch, F. Beit. z. Path. Anat. u. z. allg. Path., 1928, lxxix, 333.

EXPERIMENTAL STUDIES ON THE GLYCOGEN CONTENT OF THE PERFUSED HEART OF THE RABBIT[1]

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Received for publication April 20, 1933

This study is concerned primarily with the effect of various substances on the glycogen content of the perfused heart of the rabbit. Macpherson, Essex and Mann found it possible to cut serial sections of the perfused heart and to determine the glycogen level in these sections, without markedly impairing the activity of the remaining portion of the heart. A drop in the glycogen level was always noted in the specimens taken after the preliminary control section at the beginning of the experiment, and the use of glucose in the perfusing medium seemed to have a sparing effect on the stored glycogen. With these considerations in mind, it was determined to see if other substances might have as much or more of a glycogen-sparing effect on the myocardium. Substances such as levulose and sucrose, lactic acid and lecithin were used.

METHOD. The perfusion was carried out essentially as described by Locke and Rosenheim. The perfusion fluid was always made up fresh with glass distilled water just prior to carrying out each experiment and was maintained at about 37.5°C. Rabbits were etherized and bled by sectioning the abdominal aorta, after which the heart was removed. The heart was carefully washed with warm Ringer-Locke solution, and attached to the perfusion apparatus. The first section was taken at once through the tip of the ventricle, and at intervals of forty-five minutes thereafter similar sections were made transversely by means of a sharp razor blade. Three other sections were taken: section 2 was taken from the midventricular area, section 3 just below the auriculoventricular juncture, and section 4 included all of the heart that remained, namely, the auriculoventricular juncture and the auricles.

Excess fluid at each section was pressed out in a clean towel and the section was then weighed and placed immediately in boiling 60 per cent potas-

[1] Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine, June, 1933. Work done at the Institute of Experimental Medicine, The Mayo Clinic, under the direction of Dr. Hiram E. Essex.

sium hydroxide solution. The section became completely macerated after standing in a boiling hot water bath for an hour. Following this the glycogen was determined by the Pflüger method, modified to include the Folin method of determination of sugar.

The first substance used in the perfusing medium was glucose at a concentration of 0.2 per cent. The rate of fall in glycogen corresponded almost identically to that described by Macpherson, Essex and Mann; the greatest drop appeared in the first hour of the experiment.

A considerable number of experiments were carried out, using levulose, sucrose, lactic acid and lecithin in the perfusing fluid. The maximal amount of each substance that could be used without impairing the activity of the heart was determined in each instance and used in all experiments in the series. This was found to be 2 grams per liter. In the experiments with lactic acid 1.62 cc. of chemically pure lactic acid was added directly to the perfusion fluid after which the pH was adjusted to between 7.1 and 7.3 by the addition of sodium hydroxide. In order that an excess of sodium ions might not be present, 7.5 grams of sodium chloride were used in place of the usual 9 grams per liter in making up the Ringer solution. In using lecithin it was noted that amounts in excess of 25 mgm. to the liter of Ringer's solution seemed to impair the activity of the heart (fig. 1).

The results obtained with these substances were not significantly different from those obtained when glucose alone was used. The rapid initial drop in the level of glycogen appeared to be much the same no matter what substance was used in the perfusion fluid.

Visscher and Mulder found that the glycogen values of the hearts used in their heart-lung preparations after having functioned as long as six hours were not low. They also found that if they permitted anoxemia to develop in any of their preparations, the terminal glycogen values were apparently much lower. This suggested that the rapid initial drop of the glycogen value in hearts perfused in the ordinary manner with Ringer-Locke solution might be due to anoxemia. The logical method of combating this would be to add blood to the perfusing fluid. This has not been done in the past because of the difficulty of preventing the ultimate clotting of heparinized blood and the fact that toxic properties develop in defibrinated blood unless it passes through the lungs. Herrick and Markowitz were unsuccessful in their attempts to use defibrinated or heparinized blood in heart perfusions. It was therefore decided to see if blood cells alone added to the perfusion fluid might answer the purpose.

Rabbits were bled from the jugular veins to the extent of about 30 cc. This was heparinized and centrifuged, after which the supernatant plasma was poured off, leaving about 5 cc. of cells. After two washings in Ringer's solution the cells were added to the perfusing fluid. A marked increase in the pulse rate and vigor of the heart invariably followed, and different

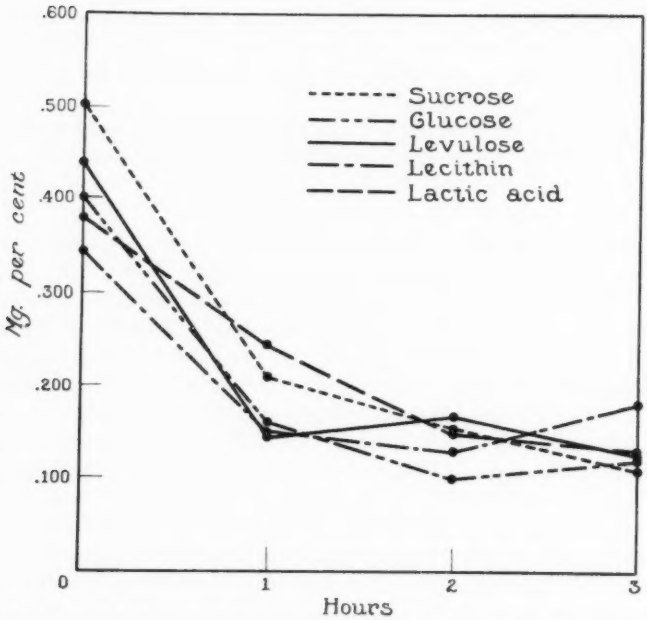

Fig. 1. Curves of glycogen fall occurring during perfusion with Ringer's solution and various substances indicated. Each curve represents the average of a number of experiments in which the same substance was used throughout.

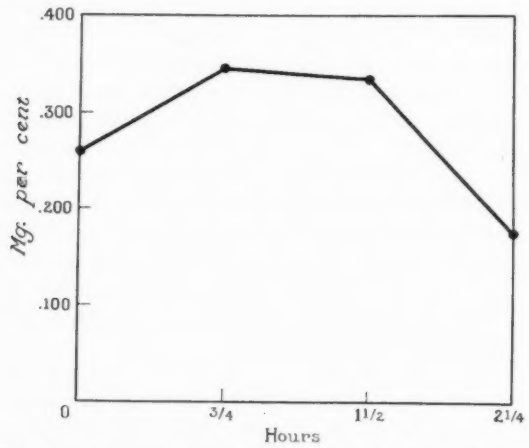

Fig. 2. The type of glycogen curve observed when Ringer-Locke solution and washed erythrocytes were used as a perfusion medium.

curves for glycogen values were obtained. The big drop noted in the first hour of the experiment was absent, and in some experiments the second section had a glycogen value even higher than in the original control section (fig. 2).

Not all curves obtained corresponded in contour with that just described, but they were all consistent in that no marked drop in glycogen values occurred in the first period of the experiment; the general tendency was for them to remain more or less constant.

Hemoglobin-Ringer's solution has been successfully used by Amberson and Höber in perfusion experiments on salivary glands. They attributed their results to the colloidal osmotic pressure of the solution approximating that of normal serum, and the lack of vasoconstricting properties, and oxygen-carrying power. They also stated that oxygen bound by free hemoglobin was not as available for the tissues as it would be if intact erythrocytes were present. From this it would appear that the presence of erythrocytes in the perfusion medium would substantially increase its oxygen-carrying capacity. Consequently the difference in the glycogen curve obtained after using the washed erythrocytes in the perfusion fluid might be explained on the basis of more adequate oxygenation.

In individual experiments, sudden decreases in glycogen content in the second and third periods are often apparent. Although cause for this was not determined, it is reasonable to suppose that in cutting sections high through the heart the opening of large coronary vessels induces anoxemia from reduced pressure of the perfusion medium in the outlying coronary circulation.

Finally, two series of experiments were carried out, using the modified perfusing fluid, one in which glucose was used and the other in which it was not used. The glycogen decreased more slowly in the hearts in which glucose was added to the perfusing fluid.

SUMMARY

Hearts of rabbits were perfused in the usual manner, with the employment of various substances in the perfusion fluid. Specimens were secured from the perfused heart at various intervals, and the glycogen content was estimated. The finding of a marked initial decrease in the glycogen content of the perfused heart was corroborated. A modified perfusion fluid was devised by the addition of washed erythrocytes to Ringer's solution, which appeared to increase the vigor and rate of the perfused hearts and prevented the initial decrease in the glycogen content of the heart muscle. On the basis of this investigation it is suggested that the decided fall in glycogen is due to anoxemia which is partially counteracted by the presence of erythrocytes. The addition of glucose to the perfusing fluid containing erythrocytes appeared to retard the depletion of glycogen from the perfused heart.

REFERENCES

AMBERSON, W. R. AND R. HÖBER. 1932. Journ. Cell. and Comp. Physiol., ii, 201.
HERRICK, J. AND J. MARKOWITZ. 1929. This Journal, lxxxviii, 698.
LOCKE, F. S. AND O. ROSENHEIM. 1907. Journ. Physiol., xxxvi, 205.
MACPHERSON, W. E., H. E. ESSEX AND F. C. MANN. 1932. This Journal, xcix, 429.
VISSCHER, M. B. AND A. G. MULDER. 1930. This Journal, xciv, 630.

THE REGULATION OF THE HEMOGLOBIN LEVEL IN POULTRY

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Received for publication April 17, 1933

That the picture of hemoglobin formation in poultry conforms in its general aspects to that characteristic of mammals has been shown by Elvehjem and Hart (1929). However, the hen differs from the mammal in one respect which may be of significance, viz., the former during long periods of time produces large numbers of eggs. Since eggs contain appreciable quantities of iron, as well as traces of copper, and since these elements are essential to the maintenance of a proper hemoglobin level, it is possible that an anemia might be associated with a heavy production. At the same time the importance of other factors concerned, such as the type of diet, the rôle of iron and copper, the possible existence of an iron reserve in spleen or liver, the number of erythrocytes, the age, sex, and condition of the birds, etc., make it quite evident that a comprehensive investigation of the entire situation is necessary if the mutual relationship between any or all of these factors is to be determined.

METHODS. Unless otherwise specified, all the hens and cocks concerning which data are reported were mature and apparently normal in all respects. Their diet, save where deviations were made in connection with particular experiments, was that which is standard for the University of California breeding flock. This diet contains as much iron and copper as is usually fed to the average commercial flock and certainly cannot be considered one calculated to induce an anemic condition in the poultry.

In every case, prior to estimating the hemoglobin, the bird was placed in a cage for one hour and kept as quiet as circumstances permitted. This was deemed advisable since it was shown by us (Harmon, Ogden, and Cook, 1932) that the spleen of the fowl acts as a reservoir for red blood cells and that any great physical disturbance is likely to be followed by the injection of this reserve into the blood stream, with consequent increase in measured hemoglobin content. After an hour of rest, the bird was removed gently and a sample of blood obtained by puncturing the vein of the wing. This treatment caused very little struggling. The hemoglobin was estimated

[1] We wish to express our deep appreciation of the keen interest and unfailing support given us by the late Dr. Walther F. Holst, Chairman of the Division of Poultry Husbandry, University of California, by whose efforts this investigation was made possible.

with a Dare hemoglobinometer of the most recent model (16 grams hemoglobin per 100 cc. blood is equivalent to 100 per cent).[2]

The normal hemoglobin value. The existing reports of measurements of the hemoglobin value of hen's blood all tend to show that the value is lower than that commonly accepted for mammals (16 to 17 gms. per 100 cc.). Our data confirm the general impression derived from previous work[3] that both in individual cases and in group averages there is an extremely wide variation in the hemoglobin value. A portion of this material is presented in table 1.

The mean values for fowls cited from the literature range from 9 to 14.7 grams per 100 cc. The two values for cocks (Dukes and Schwarte, 1931, and table 1) are 13.5 and 14.3. These figures fall within the extremes found with hens, but if we may argue from only two cases, they seem to indicate a slightly higher trend in cocks than hens. It might be supposed that the most uniform results would be obtained with young chicks before

TABLE 1

Mean and extreme hemoglobin values of poultry from the University of California Breeding Farm

TYPE OF FOWL	NUMBER OF INDIVIDUALS	MEAN Hb VALUE IN GRAMS PER 100 CC.	EXTREME Hb VALUES IN GRAMS PER 100 CC.
Group 1: Capons	5	12.4 ± 0.34	9.8–14.1
Group 2: Cocks	11	14.3 ± 0.29	10.6–16.8
Group 3: Chicks, male	64	8.2 ± 0.12	4.0–12.2
Group 4: Chicks, female	69	7.5 ± 0.10	4.4–10.2
Group 5: Chicks	606	11.9 ± 0.058	5.3–19.0

egg production could develop in the female or secondary sex characters become evident in either. The validity of the data, of course, would depend upon the members studied. The group of 115 chicks reported by Hart, Elvehjem, Kemmerer, and Halpin (1930) and the 133 chicks included in groups 3 and 4 of table 1 agree substantially with a mean value of 6 to 8 grams per 100 cc. But, on the other hand, the 600 chicks in group 5 are notably higher (mean value 11.9 gms. per 100 cc.). We are unaware of the breeding conditions and dietary background of the chicks used by

[2] In order to avoid confusion all hemoglobin values in this paper are expressed as grams per 100 cc. Different instruments have used different weights per 100 cc. as a basis (100 per cent), a situation which may be avoided by reducing in all cases to a weight and not percentage basis.

[3] Previous estimations on normal birds may be found in the work of Forkner (1929), Mack (1908), Klieneberger and Carl (1912), Burnett (1908), Schmeisser (1915), Elvehjem and Hart (1929), Hart, Elvehjem, Kemmerer, and Halpin (1929), Dukes and Schwarte (1931).

the Wisconsin workers, but have no reason to suppose they differed essentially from our own. We may class their chicks, therefore, with our groups 3 and 4, and say that three distinct groups of chicks with a total of 248 individuals have substantially the same mean hemoglobin value. There was no reason to suppose in advance that our group 5 would deviate from this general mean.

It should be pointed out in passing that the range of individual difference in group 5 is from 5.3 to 19.0 grams per 100 cc., which is even greater than similar ranges reported in the literature. If it be assumed that the upper limit represents the "normal," then the many individuals at the lower limit, if we may use mammals as a criterion, must suffer from a really profound anemia. And yet superficial examination of the flock gives no indication of any such condition. The relatively anemic chicks appear to the casual eye as perfectly normal.

Taking the available data into consideration, it seems to us futile to consider any arbitrary hemoglobin level as normal unless we wish to designate the highest obtained values as such and consider most of the hens and chicks now extant as abnormal. If such be the case, considerable study is needed to determine the factors underlying such universal abnormality. On the other hand, for *working purposes* we may in any instance define a basal level contingent upon the conditions obtaining and use this level as a point of reference for comparative investigation.

The egg production factor. Dukes, Schwarte, and Brandt (1932) state that there is no correlation between spring egg production and hemoglobin. Since this conclusion was at variance with our own preliminary observations and since it seemed unlikely on general principles, we have reëxamined the question. Our data fall in three categories: 1, the mean hemoglobin value of a number of hens throughout several months during which the average egg production varied; 2, the hemoglobin of several individual hens which passed rapidly from production to non-production and vice versa; and 3, field observations in large groups of hens, some of which were laying and some not laying.

I. A group of 17 pullets was studied during the period of February to December, 1932, inclusive. The data are clearly presented in graphical form in figure 1. The minor deviations both in the case of the egg production and that of the hemoglobin represent irregularities produced by individual birds. If a larger number could have been studied the curve would undoubtedly have been smoother. But the group tendency with respect to change in rate of egg production is unmistakable. The hemoglobin value varies inversely with the rate of egg production.

II. Certain hens occasionally cease laying even during the most productive seasons, such cessation often being very sudden. After a period of inactivity they may begin producing again at a rapid rate. These

interludes, during which the hen is said to be "broody," afford an opportunity to check the hemoglobin quite closely with the egg production. During the course of observation of a rather large flock when a given individual was noticed to be of the broody type, she was segregated and studied

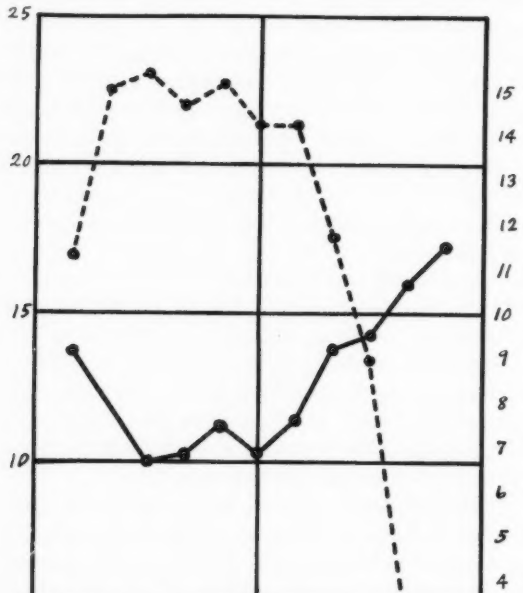

Fig. 1. Variation of hemoglobin with egg production in a group of 17 hens. The ordinate figured on the left, and the broken line represent the egg production (average eggs per hen per month) while that figured on the right, and the solid line represent the average hemoglobin level in grams per 100 cc. The abscissa shows the time, as stated, in months.

intensively. In this way data were secured with reference to 5 hens, each of which was carried through at least one complete cycle of broodiness. In fact, ten cases of broodiness were studied. In each of these no eggs were laid for a period of at least 10 days, usually much longer. Invariably the broody period was one during which the hemoglobin value rose markedly,

often to very high levels. Just as regularly the resumption of laying was associated with a fall in hemoglobin of considerable extent. The range in hemoglobin was as a rule considerable (from 6.8 to 13.6 gms. per 100 cc. in one case, and from 6.1 to 12.7 in another). In fact, the range here observed is fully as great as that found between individuals at a given time when a large flock is tested and the upper limit is approximately that found generally in the male birds.

III. Certain supplementary data were secured by random tests on 210 hens in the field, selected from four poultry farms throughout the State. This material tended to support the preceding results, for in all cases did the means indicate an inverse relation between egg production and hemoglobin level.

The influence of dietary iron. A second factor, following that of egg production, which might be expected to affect the hemoglobin level in the hen is that of the iron contained in the diet. Indeed some data already exist concerning this matter, for Elvehjem and Hart (1929) found that chickens which had been made extremely anemic by the feeding of a diet abnormally low in iron might be restored to their previous condition by the readministration of the metal. Thus they reduced the average hemoglobin level from about 7 grams per 100 cc. to 4 grams per 100 cc. and then restored the former level. They did not, however, raise the value above the starting point. Although their work undoubtedly demonstrated the efficacy of iron feeding in the building up of hemoglobin, it seemed to us worth while to attempt once more the raising of the hemoglobin level in birds which had *not* been subjected to a deliberately induced anemia. That is, let us assume that the ordinary, standard ration is deficient in iron for many birds, and that the latter with hemoglobin values of 7 to 10 grams per 100 cc. are really somewhat anemic. Then, even despite Elvehjem and Hart's reported results, if their general theory is correct, as it is universally conceded to be, it should be possible to raise the hemoglobin value if enough excess iron is fed. In their investigation they fed only a few milligrams per cent of iron. To make certain that we did not err on the side of too small a dose we gave approximately 250 mgm. or more per 100 grams of food. In addition, we tested the efficacy of liver extract in a similar way.

We can report positive results with three separate experiments, performed at different times and on different groups of birds.

I. The mean hemoglobin values were studied in three groups of six hens each. One group was fed liver extract, one ferrous sulphate, and one group was maintained on the standard diet with no addition. Although there was considerable minor fluctuation, as indicated by the control group, due probably to individual variations and to the relatively small number of hens investigated, nevertheless certain features were clearly

apparent: 1. As contrasted with the controls, both the experimental groups showed a slight but definite response to liver extract, with a clear fall in hemoglobin, subsequent to omitting this material from the diet. 2. There was a strong response whenever ferrous sulphate was added, followed by a definite fall in hemoglobin when the iron was discontinued.

II. To test the effect of feeding iron to large numbers, a group of 125 chicks just out of the shell was used. After estimating the hemoglobin of all the individuals, the group was split into two lots of 64 and 61 chicks respectively. The first lot was fed the standard diet plus 0.25 per cent ferrous sulphate. The other lot did not receive the additional iron. At the end of four weeks the hemoglobin was again estimated. During the period the mean hemoglobin value of the lot without iron fell from 10.7 to 9.6 grams per 100 cc., whereas that of the other lot rose slightly from 10.8 to 11.0 grams per 100 cc. The difference between the lots at the start was thus about 1 per cent, but at the end it was about 13 per cent. There seems to be little doubt that here the administration of iron was of influence in maintaining the hemoglobin.

III. To substantiate experiment II (above) and to secure more detailed data, two groups of 25 newly hatched chicks were selected. One of these was fed 0.5 per cent ferrous sulphate, the other received no additional iron. At the end of 16 weeks the iron supplement was reduced to 0.25 per cent. Both groups were observed for 24 weeks, or until the laying period began. The two groups started at nearly the same level, viz., an average of 11.4 and 12 grams per 100 cc. respectively for the controls and experimental birds. There followed a rapid divergence during the growing period, reaching a maximum in 12 weeks. The difference then was 10.4 to 8.5 grams per 100 cc. Subsequently, the values converged until at the end of 24 weeks they were substantially equal (9.9).

Other considerations enter here. The experimental group was beginning to lay at the end of 24 weeks. Actually 6 of the 25 pullets were laying, as compared to one of the control group. The average hemoglobin level of the *non-laying* birds of the iron fed group was 10.3 grams per 100 cc. and that of the *non-laying* controls was 10.0, a slight but distinct difference. The average value for the six laying, iron fed birds was 8.8 and the value for the one laying control was 7.8. Although too much weight should not be placed on data involving so few birds, nevertheless the uniform tendency for the iron fed birds to maintain a higher hemoglobin level is unmistakable. Furthermore, the tendency for the iron fed birds to begin laying earlier than the controls must be of significance. Taken in conjunction with the results previously reported, it affords a reasonable explanation for the fall in hemoglobin value of the iron fed group prior to the 24th week.

While these experiments in themselves cannot be considered absolutely

conclusive, they create a very strong presumption that hens fed the standard diet may undergo an increase in hemoglobin when extra iron is fed them. In any case it makes necessary the inclusion of dietary iron among the factors concerned with the maintenance of a proper hemoglobin value in poultry.

The splenic reservoir factor. We have previously shown (Harmon, Ogden, and Cook, 1932) that the spleen of the hen resembles that of the mammal in that it may act as a reservoir for red blood cells. This reservoir empties in time of stress and injects the stored erythrocytes into the blood stream, thereby increasing the number of oxygen carriers. Both acute and chronic conditions of oxygen deficiency may serve as stimuli for the contraction of the spleen. We might, therefore, expect that those hens which have at a given time a relatively high hemoglobin level would also show a relatively large splenic reserve, and conversely when the hen becomes markedly anemic from whatever cause, that the reserve would be depleted. There would, therefore, be a positive correlation between the hemoglobin level and splenic reserve.

In the course of investigation of other factors a number of determinations of the spleen reserve were made. This was done by first estimating the hemoglobin after a period of quiet (the standard procedure in all cases) and then, after occluding the trachea until asphyxia was well advanced, making a second estimation. Since asphyxia, with its concomitant muscular effort, is one of the most powerful stimuli known to the emptying of the splenic reservoir, it may be assumed that such treatment ensures the maximum effect. The difference between the two readings is, then, a fairly accurate index to the capacity of the spleen in increasing the hemoglobin level. For purposes of comparison the hens tested were arbitrarily separated into two groups, depending on whether the resting hemoglobin value was below or above 8.0 grams per 100 cc. Although all such data are relative, this value may be taken as approximately the point below which the hen becomes definitely anemic and below which one might expect to find a permanent diminution of the reservoir capacity of the spleen as indicated by its relative inability to raise the hemoglobin level after asphyxia.

Of the 279 hens tested the high hemoglobin group showed a mean increase in grams per 100 cc. blood of 1.16 ± 0.034 and the low hemoglobin group one of 0.54 ± 0.026. Here the difference of the means equals $1.16 - 0.54$, or 0.62 and the error of the difference $\sqrt{0.026^2 - 0.034^2}$ or 0.0428. The ratio of the difference to the error of the difference is therefore 14.5, which establishes the difference as significant.

It seems therefore apparent that in considering the depletion of the hemoglobin supply because of nutrition deficiency, egg production, or for any other protracted cause we must include the splenic factor. This

factor would, of course, tend to operate *counter* to the forces bringing about the anemia, and to a certain extent would mitigate its severity.

The iron storage function of the spleen. Previous work in this laboratory (Cook and Spilles, 1931) has indicated that in rats the stored iron in the spleen may be withdrawn and utilized during periods of dietary anemia. This utilization, however, was shown to be dependent upon the presence of an adequate supply of copper. Since we have not yet had the opportunity to investigate the rôle of copper in the hemopoiesis of the hen, we have attempted here only an orienting survey of the splenic iron storage function with a view to examining the situation under more or less "normal" conditions. The following account, therefore, is of the nature of a preliminary statement.

The spleens were in all cases removed after bleeding and dried to constant weight. They were then ashed and analyzed for iron colorimetrically with thioglycollic acid.

In table 2 are grouped data pertaining to the splenic iron and spleen size in newly hatched and growing chicks. From these data it is at once apparent that the chick on hatching possesses a spleen of extremely small size, since the average dry weight of fifty spleens is only approximately 3 mgm. If we assume that the dry weight of the organ is about one-fifth of the fresh weight, and that the average newly hatched chick weighs about thirty grams, then the spleen represents only 0.05 per cent of the body weight. The total iron content is correspondingly small and furthermore, as we determined by analysis, the relative iron content $\left(\frac{\text{mgm. Fe}}{\text{gm. spleen}}\right)$ is no greater than that of other tissues. The chick, therefore, in contradistinction to the mammal, starts its independent existence with no stored iron whatever. (In the spleen of the newly born rat the relative iron content may be five times as great as that in the adult spleen.) Within a month after hatching definite changes occur in the chick (see table 2, lots A to D). Particularly noticeable is the great increase in spleen size as contrasted with that in the relative iron content. Continued growth, up to twelve weeks, is accompanied by further increase in spleen size, but the relative iron content seems to reach a maximum at about $1.1 - 1.2$ $\frac{\text{mgm. Fe}}{\text{gm. spleen}}$. Considering the birds on standard diet only (lots C, E, and G in table 2) the increase in spleen weight is more than one hundredfold, whereas the increase in relative iron content is slightly more than twofold. Thus in the young bird under these conditions the relative splenic iron content appears to be held at a level which cannot be greatly in excess of the relative iron content of the tissues at large. As a matter of fact, if these data represent the true state of affairs, it becomes very difficult to evaluate the iron storage function of the spleen in terms of iron *concen-*

tration in that organ. If, then, the relative iron content is approximately constant, the absolute size of the spleen becomes the critical factor in considering the amount of iron which might be made available. The enormous growth of the organ has already been pointed out. That this growth, however, is subject to modification will be seen from those chicks which were fed a high iron ration as contrasted with those not so fed (see lots D, F, and H in table 2). Both groups run closely parallel with respect to

TABLE 2

The weight and iron content of chicken spleens

LOT	NUMBER OF CHICKS	AGE IN DAYS	DIET	AVERAGE DRY WEIGHT OF SPLEEN IN MILLIGRAMS	AVERAGE TOTAL WEIGHT OF IRON PER SPLEEN IN MILLIGRAMS	AVERAGE RELATIVE AMOUNT OF IRON IN SPLEEN: MGM. Fe / GM. SPLEEN	REMARKS
A	50	1	—	3	0.0016	0.53	All spleens dried, weighed and analyzed in one lot
B	50	3	Standard	2.6	0.002	0.77	Standard diet is that ration provided the general breeding flock at California
C	10	28	Standard	19.2	0.0143	0.74	
D	10	28	Standard plus iron	13.7	0.0125	0.91	Iron added as $FeSO_4$ up to 0.25 per cent. Added at 7 days age
E	10	56	Standard	88.9	0.097	1.1	
F	10	56	Standard plus iron	74.3	0.081	1.09	Iron added as $FeSO_4$ up to 0.25 per cent. Added at 7 days age
G	10	84	Standard	364.3	0.422	1.16	
H	10	84	Standard plus iron	154.1	0.177	1.15	Iron added as $FeSO_4$ up to 0.25 per cent. Added at 7 days age

the relative iron content, and hence amount of iron in the diet, as such, although it may influence the hemoglobin level, has no effect on the splenic iron level. But the spleen size varies widely. The low iron chicks at twelve weeks show spleens more than twice as heavy as those of the high iron group. Concerning the significance of this we have no comment to make at present beyond emphasizing again that the influence of the dietary iron, if any, is exerted through modification of spleen size rather than splenic iron concentration.

We also investigated the situation in *adult* hens. We brought together data concerning the hemoglobin value of hens prior to killing, the previous history of egg production, the spleen size, and the iron content, both total and relative. The diet of all these hens was standard. However, inspection of the data as well as statistical treatment, revealed no clear correlation between any of these factors, except between spleen size and total iron content. Here the coefficient of correlation was 0.716 ± 0.073. This, of course, simply substantiates the finding in the previous case that the relative iron content of the spleen is very nearly constant (and at a value tending to approach $1.1\text{–}1.2 \frac{\text{mgm. Fe}}{\text{gm. spleen}}$). The correlation between spleen size and hemoglobin value was too slight to be of significance. A comparison of spleen size, total iron content, and relative iron content with respect to the heaviness of egg laying failed to show any significant differences.

A somewhat different approach to the question of the splenic iron content was based upon the fact that in mammals much of the splenic iron is stored in the form of hemosiderin granules. Using the technique described in a previous paper (Cook, 1929) we investigated the spleen of the hen. The results were quite definite in the negative sense. No hemosiderin could be detected with the microscope in sections of several spleens. Acidified potassium ferrocyanide gave no blue color whatever beyond a very feeble diffuse tint referable to contamination by the iron of the sectioning knife. Digestion of a spleen with hot 30 per cent hydrochloric acid followed by potassium ferrocyanide gave only a slight bluish color, probably due to the small residue of blood in the spleen. No material concentration of iron in the spleen was therefore to be detected by this method.

SUMMARY AND CONCLUSIONS

1. An examination of a large number of chicks and hens shows that the amount of hemoglobin in the blood varies between very wide limits (approximately 5 to 19 gms. per 100 cc.) in apparently normal birds. This wide fluctuation indicates a flexibility in the hemopoietic system of the bird not encountered in the mammal and suggests that the hemoglobin level at any time is the resultant of a number of factors which may be operating.

2. One of these factors is shown to be the intensity of egg production. The hemoglobin level falls with high egg production, and vice versa, such changes following either seasonal cycles or temporary variations.

3. Evidence is adduced to show that both adult hens and young chicks may undergo a marked increase in hemoglobin level when iron (as $FeSO_4$) is added to the standard diet. Such an increase is noted in birds not previously made anemic by feeding an unusually low iron ration.

4. The blood reservoir function of the spleen is effective in the sense that the capacity of the splenic reservoir is reduced in relatively anemic birds,

indicating a state of permanent partial contraction of the spleen. The effect of this action would be a tendency to mitigate the severity of the anemic condition.

5. An iron storage function on the part of the spleen could not be clearly demonstrated and until further investigation is completed its existence must be held doubtful. In the newly hatched chick the size of the spleen is insignificant and its iron storage capacity negligible. During growth the spleen size increases tremendously, but its relative iron content rises only to about double its initial value and remains there essentially unchanged. In the adult neither the spleen size nor its iron content could be correlated with either the hemoglobin level or the production of eggs. It may be suggested, therefore, that since the hen apparently has not at its disposal a store of reserve iron in the spleen for hemopoiesis, as does the mammal, it is forced to depend almost wholly upon the current intake of iron in the diet. If this be true, that there is no stabilizing depot of Fe in the spleen, and that the dietary iron may be (and at times certainly is) subject to wide variation, we may find here a possible explanation of the great fluctuation of hemoglobin level in so-called normal hens. For the very power to alter that level, without obvious harm to the bird, may in itself constitute an adaptation to the possibility of a continually shifting supply of dietary iron.

ACKNOWLEDGMENT. We wish to acknowledge with appreciation the financial assistance rendered during this investigation by the Board of Research of the University of California.

REFERENCES

BURNETT, S. H. 1908 The clinical pathology of the blood of domesticated animals. Taylor & Carpenter, Ithaca, N. Y.

COOK, S. F. 1929 Journ. Biol. Chem., lxxxii, 595.

COOK, S. F. AND N. M. SPILLES. 1931. This Journal, xcviii, 626.

DUKES, H. H. AND L. H. SCHWARTE. 1931. This Journal, xcvi, 89.

DUKES, H. H., L. H. SCHWARTE AND A. E. BRANDT. 1932. Science, N. S., lxxv, 25.

ELVEHJEM, C. A. AND E. B. HART. 1929. Journ. Biol. Chem., lxxxiv, 131.

FORKNER, M. D. 1929. Journ. Exp. Med., l, 121.

HARMON, I. W., E. OGDEN AND S. F. COOK. 1932. This Journal, c, 99.

HART, E. B., C. A. ELVEHJEM, A. R. KEMMERER AND J. G. HALPIN. 1929. Poultry Science, ix, 92.

MACK. 1908. Quoted by BURNETT, 1908.

KLIENEBERGER, W. C. AND W. CARL. 1912. Die Blutmorphologie der Laboratoriums Tiere. J. A. Barth, Leipzig.

SCHMEISSER, H. C. 1915. Johns Hopkins Hosp. Repts., xvii, 551.

THE ELASTICITY OF VEINS

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Received for publication May 31, 1933

The coefficient of elasticity of any body is a measure of its resistance to deformation and is defined as the stress, tending to restore it to its original condition, divided by the strain. The coefficient of linear stretch or Young's modulus, which will be called M, is equal to $FL/a\,dL$, where F is the force applied, a is the area of the cross section, L is the original length, and dL is the change in length. In the case of metal wires M is constant, within certain limits, for any given material. For rubber, M is fairly constant for a time and then increases sharply with increasing force with the result that the material becomes harder to stretch. In the fibro-elastic tissues of the body M, the resistance to deformation, increases with increasing force (1). In organs containing both muscular and fibrous tissue the variation of M with the force applied is more complicated, since muscular tissue has a different coefficient of elasticity with different degrees of muscle tone or tension. In a section of an artery M increases steadily with F, if the muscular tissue is relaxed, but, if it is in a state of tonic contraction, M decreases with increasing F, that is, the wall stretches more easily with increasing pressure, until the muscular tension is overcome and the artery reaches its normal size in a relaxed condition. After this point is reached the resistance to extension is determined by the fibrous tissue present and M increases with further increase in applied force (2), (3).

In order to determine the elasticity of tissue one can take strips, measure the extension with a known weight, apply the formula for Young's modulus, and determine M directly. It is also possible to take a whole organ, the eye, the bladder, a section of the intestines or of a blood vessel, and measure the change in volume with change in internal pressure. In this case the stress/strain $= dP/\frac{dV}{V} = V/\frac{dV}{dP}$ which we may call E, the coefficient of functional elasticity of the organ in question. This is not Young's modulus M but in some cases is proportional to it. The volume change divided by pressure change (dV/dP) has frequently been determined (2), (3), (4), (5) but unless the results are related to the original volume V they have only a qualitative significance.

Some recent experiments in which the volume changes with pressure were related to the total volume were made in the cerebrospinal fluid system (6), (7). Changes in volume with pressure in the cerebrospinal fluid were related to the total intradural volume. This gave consistent values of E in animals of different size and an average value of $E = 4.2 \times 10^5$ dynes per sq. cm. in dogs, over a pressure range of 150 to 350 mm. saline. The volume-pressure changes should probably have been related to the volume of the cerebrospinal fluid instead of to the total intradural volume. The volume of the cerebrospinal fluid is difficult to determine accurately but may be taken to be 10 per cent of the total intradural volume. This would give average values for E equal to 0.42×10^5 dynes per sq. cm. for dogs, 0.35×10^5 dynes per sq. cm. for cats and 0.44×10^5 dynes per sq. cm. for macaques. In the case of the intradural system many elastic elements are present (membranes and vascular bed) and the system is too complex to relate the results accurately to the coefficient of linear stretch of any of them.

In the eye, which may be considered a sphere, M the average coefficient of linear stretch of the eye coats $= \frac{3r}{2t} V/\frac{dV}{dP} = \frac{3r}{2t} E$, where r is the radius of the eye and t the average thickness of the eye coats. Experiments on the volume and pressure changes in several different species (1) showed that r/t is a constant for eyes of different size in any one species so that M (Young's modulus) is proportional to E the coefficient of functional elasticity in the eye.

In a cylinder with elastic sides $M = \frac{2r}{t} V/\frac{dV}{dP} = \frac{2r}{t} E$, provided the assumption is made that the cylinder changes in radius with increase in pressure, while the length remains constant. A blood vessel, under pressure, changes in length as well as radius but, since the vessel is long in proportion to its width, the percentage change in length is small and this formula has been generally used for the elasticity of the vessel walls (8), (9).

In this investigation sections of veins and arteries from dogs and veins from fresh human autopsy material were placed in a small plethysmograph, filled with normal saline, and the change in volume with pressure determined over a pressure range of 10 to 60 mm. Hg. Measurements of the ratio $2r/t$ were made and E and M were determined by substitution in the above formula. Also a method was developed for determining E, the coefficient of functional elasticity in vivo, the results of which check in a satisfactory way with in vitro measurements up to 40 mm. Hg pressure.

METHOD FOR DETERMINING FUNCTIONAL ELASTICITY (E) IN VEINS IN VIVO. With the subject lying, so that vasomotor tone played as small a rôle as possible, the hand and forearm were inserted in a water plethysmograph and a pressure cuff was placed on the upper arm. The venous

pressure in the hand, in the recumbent position with the hand at heart level, is about 10 mm. Hg or less. As the cuff pressure is increased the venous pressure rises to the cuff pressure in 15 to 30 seconds (10). The pressure in the cuff, as measured by the attached mercury manometer, can therefore be taken as the pressure in the veins. A piece of rubber tubing on the plethysmograph fitted snugly around the arm but, to prevent bulging of the rubber with pressure changes, a hinged wooden disk, with a hole the size of the arm, was placed around the arm and drawn up against the end of the plethysmograph by four rods with screw ends passing through a similar disk at the other end of the plethysmograph. The small space between this disk and the rubber on the arm was filled with plasticene. The plethysmograph was swung from a support so that it followed any slight involuntary arm movements and there was no error from relative movements of the arm and plethysmograph. The water in an attached burette, brought back to the same level at each reading, gave volume changes in cubic centimeters.

In making a series of readings the pressure was raised to 20 mm. Hg for the first reading and the volume change determined for each increment of 10 mm. Hg up to 60 mm. venous pressure. Volume readings were made 15 to 20 seconds after each pressure increment. This insured complete venous volume change for each pressure change and at the same time kept the error due to filtration of fluid a minimum (11), (12). After the first run or so the arm returned to its original volume, on removal of pressure, to within a few tenths of a cubic centimeter so that any considerable error from this source was avoided but the readings above 50 mm. Hg are certainly affected by filtration to some extent, especially in young subjects. From five to ten runs were made and averaged.

These readings of volume change with pressure change gave dV/dP but, in order to calculate $E = V/\frac{dV}{dP}$, it was necessary to estimate V, the volume of venous blood in the arm. When pressure was applied on the outside of the arm in the plethysmograph, through the burette, in order to collapse the veins, it was found that for six subjects, with total arm volume in the plethysmograph ranging from 500 to 700 cc., the arm volume decreased 2.5 to 3.0 (average 2.8) per cent with an external pressure of 20 mm. Hg and 3.5 to 3.6 per cent with an external pressure of 30 mm. Hg. It was therefore assumed that at 20 mm. Hg, the starting point of the experiments, the venous volume was approximately 3 per cent of the total arm volume. Knowing V and dV/dP, the coefficient of functional elasticity, E, could be calculated and, with volume expressed in cubic centimeters and pressure in centimeters of Hg, the results multiplied by 980 $\times$ 13.6 (density of mercury) gave E in dynes per square centimeter.

The coefficient of linear stretch M will be proportional to E only if

$2r/t$ (diameter/thickness) of vein wall is a constant for veins of different size. No published results could be found on the dimensions of veins but $2r/t$ has been found to be fairly constant in the arterial system. Hürthle (13) states that, in the dog, the wall thickness is 15 per cent of the radius in all arteries, except a portion of the aorta, or $2r/t = 13$.

To corroborate this point, measurements of $2r/t$ were made on veins and arteries in a dog, injected with gelatin at 150 mm. pressure. The ratio $2r/t$ was found to be equal to 12 in arteries ranging from 1 to 3 mm. diameter, when the external diameter was taken as $2r$. This agrees well with Hürthle's figure. In veins, from 1 to 10 mm. diameter, $2r/t$ was equal to 50. Subsequently measurements were made on human autopsy material. Sections of the vena cava and iliac vein gave values of $2r/t$ ($2r$ = internal diameter) ranging from 18 to 26 in different individuals but the same in two veins from the same body. Young cases gave values from 18 to 21 (average 20) and old cases 22 to 26 (average 23.5). It is evident that although $2r/t$ may increase with age and vary in different individuals it is

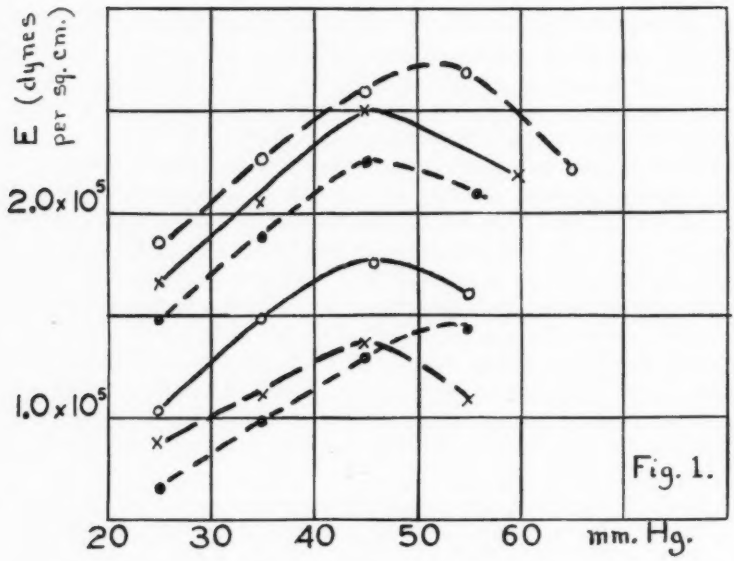
TABLE 1

dP	AVERAGE dV	dV/dP	$V/\frac{dV}{dP}$	E	$\frac{2r}{t}$	M
cm. Hg	*cc.*			*dynes per sq. cm.*		*dynes per sq. cm.*
2–3	1.60	1.60	12.5	1.67×10^5	20	3.34×10^6
3–4	1.30	1.30	15.4	2.05×10^5	20	4.10×10^6
4–5	1.05	1.05	19.0	2.53×10^5	20	5.06×10^6

constant enough in any one subject to justify the assumption that E is proportional to M.

RESULTS ON ELASTICITY OF VEINS. *In vivo.* A series of typical results is given in table 1. In this subject $V = 3$ per cent of total arm volume = 3 per cent of 665 cc. = 19.95 cc.

Thirty subjects from 12 to 73 years old were used and the results for six subjects between the age of 30 to 36 years are given in figure 1 and show a wide individual variation in E (resistance to extension). This might be due to a difference in the vasomotor tone of the vessels, in $2r/t$, in venous volume, in the relative volume of venous blood and tissue fluid, or to a constitutional difference in the coefficient of linear stretch M of the vein walls. The subject in this group with the lowest value of E had suffered from varicose veins from the age of 18 and it was thought possible that a low value of E (low resistance to volume change under pressure) might have been a predisposing cause. So far E has not been determined on other young individuals with varicosities but experiments on such subjects might be of special interest.



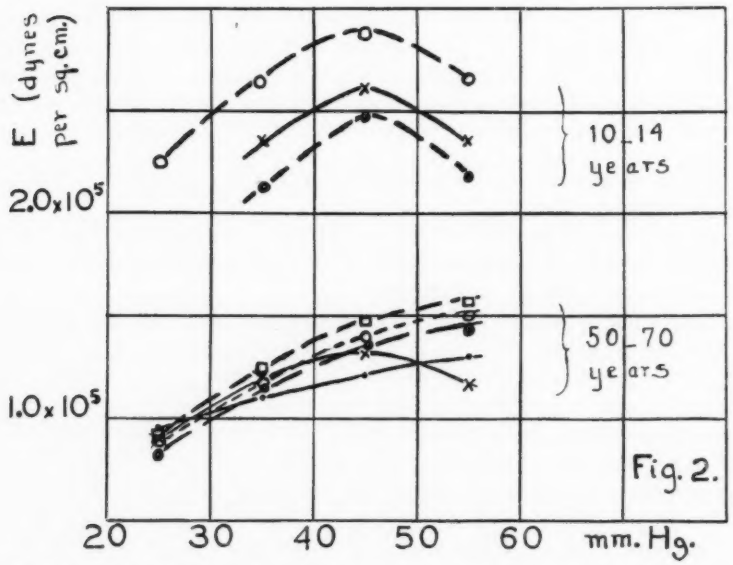


Fig. 1. *E* in dynes per square centimeter over a pressure range of 20 to 60 mm. Hg for 6 subjects (age 30–35 years).

Fig. 2. *E* (dynes per sq. cm.) for 3 subjects age 10 to 14 years and for 5 subjects 50 to 70 years old.

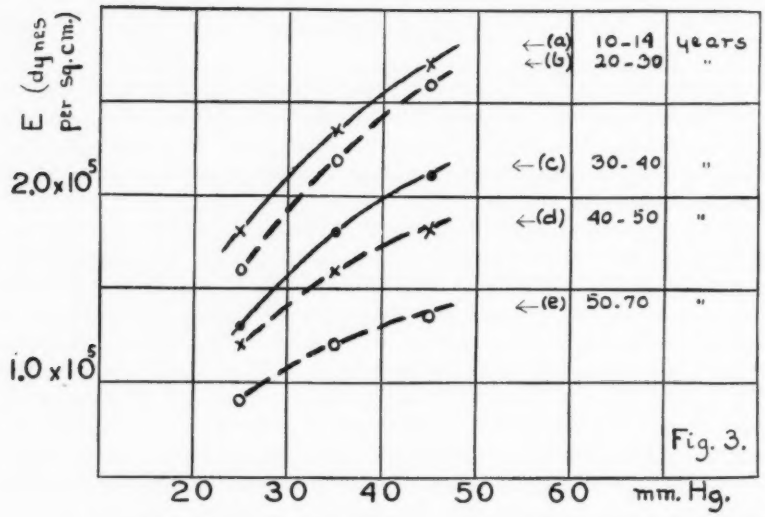
The decrease in E with pressure beyond 50 mm. Hg is probably due to the fact that at high pressures the increase in filtration rate makes the volume readings too high and lowers the value of E. The readings above 50 mm. Hg are therefore unreliable, and those from 40 to 50 mm. are probably somewhat too low.

The variation in E seems to be a real physiological difference as any one individual gave consistent results when measurements were repeated at intervals. Marked individual variations were chiefly apparent in subjects between 30 and 40. Young individuals always gave a high value of E and subjects over 50, with one exception, a low value. This is shown in figure 2 where results are given for 3 subjects 12 to 14 years old and for five subjects over 50. Also, in young subjects, the drop in E at pressures over 50 mm. Hg was always marked, which probably indicated rapid filtration at these pressures. In the old group this drop was absent except in one case and subsequent experiments showed remarkably rapid filtration in this subject. Owing to this complication, values for E should only be determined, by this method, up to 40 mm. Hg.

In spite of individual variations the results, when averaged by decades, show a progressive change in E which is high in youth and low in old age (see fig. 3). This decrease in E (increase in extensibility) with age might be due, as stated above, to a number of causes. In order to find out if it is due to a decrease in M (coefficient of linear stretch) with age, as was found in the eye coats (1), volume-pressure changes were measured on sections of the vena cava and iliac vein from fresh autopsy material over an age range from 17 to 62 years.

Elasticity of veins in vitro. Ten cases were studied and showed some individual variation but no marked age change. The values for E for the four youngest and four oldest cases are averaged in figure 4a. The older group showed a slightly lower E, especially at higher pressures. The value of r/t was found to vary from 19 to 26 with a rather clear indication of higher values in older cases. When M was calculated it was found to be identical in the two groups (see fig. 4b) so that the coefficient of linear stretch did not change with age in these veins and the slight difference in E between the young and old groups was due to an increase in $2r/t$ with age. Veins habitually subjected to greater pressures, such as the veins of the leg, might have shown an age change in M similar to that found in the eye.

Discussion. The results on autopsy material checked very well with the values found for young subjects in vivo over a pressure range from 20 to 40 mm. Hg. The low values found for the functional elasticity E in all subjects over 50, and in some as young as 35 years, are apparently not due to a change in M. They may be accounted for in part by a change in vein dimensions and vein volume with age but another factor, which would influence the swelling of veins under pressure, is the resistance offered to



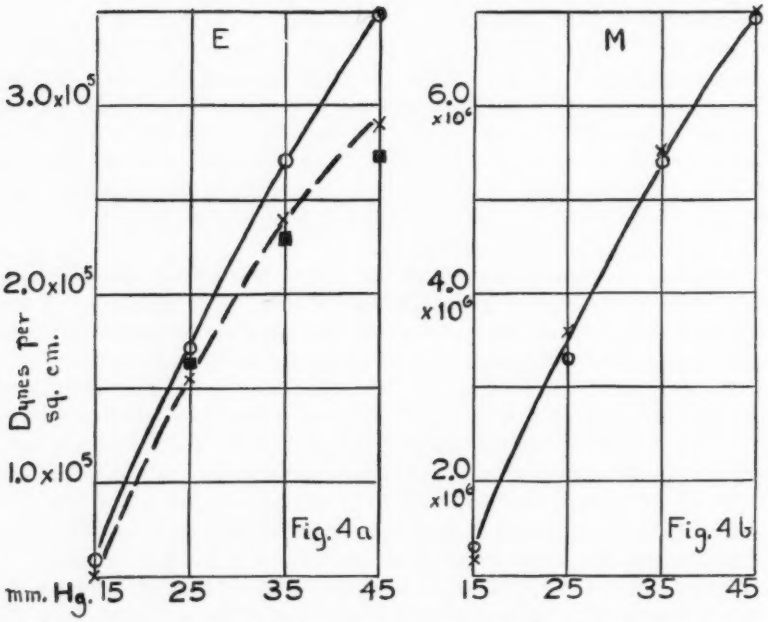


Fig. 3. Average value of E (dynes per sq. cm.) in different age groups. (a) 3 subjects 10 to 14 years, (b) 7 subjects 20 to 30 years, (c) 6 subjects 30 to 40 years, (d) 3 subjects 40 to 50 years, (e) 6 subjects 50 to 70 years.

Fig. 4 (a) E (dynes per sq. cm.) measured on excised veins. 0——0 4 young cases, X——X 4 old cases: ■ ■ Average values with living subjects age 15 to 30 years.

Fig. 4 (b) M (dynes per sq. cm.) excised veins; 0——0 4 young cases: X——X 4 old cases.

extension by the tissues surrounding the veins. An attempt was made to determine the relative volume of venous blood and tissue fluid by measuring the volume change in both for the same pressure change. If V_1 is the volume of venous blood, V_2 the volume of tissue fluid, and dV_1 and dV_2 the changes in volume for the same pressure change dP, then $V_2/V_1 = F\ dV_2/dV_1$ where F is a function of the elasticity both of the vein wall and of the skin.

Measurements of dV_2/dV_1 were made on nine individuals who had been subjects in the experiments for the determination of E. Previous work on change in arm volume with continued pressure (11) has shown that there is no filtration for venous pressures less than 20 cm. water. When venous pressure is raised to a point at which considerable filtration takes place (for instance 40 mm. Hg), the change in venous volume takes place rapidly, being complete in 15 to 30 seconds. This first rapid rise is followed by a slower rise for 6 to 8 minutes, after which the rate of increase becomes very slow. The second rise has been interpreted by some authors (12) as being due to relaxation and filling of capillaries and venules and by others as due to filtration. It seems reasonable to assume that when an internal pressure of 40 mm. Hg is put on the veins, the immediate rise in arm volume is due to a change in venous volume and that the slower rise between 30 seconds and 6 minutes is due to filtration, which is rapid as long as there is a pressure difference between the blood vessels and the tissue fluid. As the tissue fluid volume increases an approximate pressure equilibrium is reached (at 6 to 8 minutes) and the slow rise after that is due to slow filtration under conditions approaching equilibrium. With these assumptions, the following experiment was performed. With the arm of the subject in the plethysmograph, the pressure in the pressure cuff was raised to 15 mm. Hg and kept at this pressure for a minute. The level in the burette remained constant, after a small initial rise, as this was near or below capillary pressure and there was no filtration. Ideally, this initial pressure should be determined for each subject so as to be at the level where filtration is just avoided. The pressure was then raised to 40 mm. Hg and the immediate rapid rise in volume read at the end of 15 to 30 seconds. This gave the change in venous volume. The pressure was then kept at the same level for 6 to 8 minutes, volume readings being taken every minute. When approximate equilibrium was reached, which was taken to mean approximate pressure equilibrium between the blood and tissue fluid, the change in volume between the 30 second and 6 minute readings was taken as the change in volume of the tissue fluid. If, during this time, the pressure in the cuff had fallen to 37 or 38 mm. Hg the change in venous volume was corrected to this level.

The value of dV_2/dV_1 varied from 0.6 to 2.3. The average values, based on this small group, were 2.1 for the 20 to 30 year old subjects, 1.65

for those 40 to 50 years old, and 1.2 for those over 50. This may be due to a change in F with age or may indicate a larger volume of tissue fluid in young subjects. Drinker (14) found more lymph in young than in old dogs, which he believed due to a decrease in capillary permeability with age. If there is a decreased formation of tissue fluid, and consequently smaller tissue fluid volume and lower tissue fluid pressure, in old age, this would account for the decrease in functional elasticity of the veins.

Bramwell and Hill (9), from measurements of pulse wave velocity, found that the extensibility of arteries decreases with age. This may be due to calcification of the artery wall but is probably due in part to a change in the ratio $2r/t$. Arteries show an increase in circumference and also in wall thickness with age (5), (15), (16). It is interesting that the changes in functional elasticity in veins and arteries with age are in opposite directions.

TABLE 2

Comparative values of E *and* M *(in dynes per sq. cm)*

PRESSURE	DOG		HUMAN (IN VITRO)		
	E	M	E	M	
cm. Hg					
1–2	1.21×10^5	1.45×10^6			Arteries
2–3	1.4×10^5	1.68×10^6			
3–4	1.4×10^5	1.68×10^6			
1–2	0.15×10^5	0.75×10^6	0.53×10^5	1.2×10^6	Veins
2–3	0.28×10^5	1.4×10^6	1.63×10^5	3.5×10^6	
3–4	0.42×10^5	2.1×10^6	2.55×10^5	5.4×10^6	

As arteries become less extensible veins become more extensible. This would decrease the effective volume of circulating blood.

COMPARATIVE VALUE OF E AND M IN DIFFERENT SPECIES. Results obtained on the veins and arteries of dogs and on the veins in human subjects are given in table 2. In the human subject M for veins is about twice as great as in the dog (see table 2).

CONCLUSION

A method is described for determining E, the coefficient of functional elasticity, of veins in vivo. The results were checked by measurements of E and M (coefficient of linear stretch) on veins from autopsy material. The values of E are subject to wide individual variation and show a progressive fall with age. The decrease in E (increase in extensibility) with age is not due to a change in M as this is found to be the same in different age groups. The increased extensibility is thought to be due in part to a decreased production of tissue fluid in older subjects.

REFERENCES

(1) Clark, J. H. This Journal, 1932, ci, 474.
(2) MacWilliams, J. A. Proc. Roy. Soc., 1902, lxx, 132.
(3) Kesson, J. E. Quart. Journ. Physiol., 1913, vi, 355.
(4) Roy, C. S. Journ. Physiol., 1880–82, iii, 125.
(5) Strasburger, J. Deutsch. Arch. f. Klin. Med., 1907, xci, 378.
(6) Flexner, L. B., J. H. Clark and L. H. Weed. This Journal, 1932, ci, 292.
(7) Weed, L. H. and L. B. Flexner. This Journal, 1932, ci, 668.
(8) Frank, O. Zeitschr. f. Biol., 1920, lxxi, 255.
(9) Bramwell, J. C. and A. V. Hill. Proc. Roy. Soc. B. 1922, xciii, 298.
Ibid. 1923, xciv, 450.
Heart, 1923, x, 233, 289.
(10) Lewis, T. Heart, 1924, xi, 109.
(11) Krogh, A., E. M. Landis, and A. H. Turner. Journ. Clin. Invest., 1932, xi, 63.
(12) Drury, A. N. and N. W. Jones. Heart, 1927, xiv, 55.
(13) Hürthle. Quoted from Tigerstedt, Physiologie des Kreislaufes.
(14) Drinker, C. K. and M. Field. This Journal, 1931, xcvii, 32.
(15) Grunstein, N. Arch. Micros. Anat., 1896, xlvii, 583.
(16) Schiele-Wiegandt, V. Virchow's Arch., 1880, lxxxii, 27.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XXXII. Basal Metabolism and the Temperature Factor in Brooding Ring Doves

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Received for publication May 19, 1933

The reproductive cycle in doves and pigeons involves a sequence of fairly rapid and well-marked changes in the behavior, endocrine-status and body weight of these animals. The various phases of this cycle therefore here invite a study of the question whether and to what extent cyclic variations in basal heat production occur within the (reproducing) individual. The profound changes in behavior of these birds associated with broodiness or incubation—in which the male here takes full part—is known to all. The mobilization of a considerable part of the female endocrine system at the time of ovulation has been reported by Riddle (1), (2). That temporary body growth cyclically occurs in the individual adult during the period of incubation, with a recession of body weight during immediately subsequent feeding of the young, is partly indicated by data incidentally reported here and more completely shown by data soon to be reported by Riddle and Braucher (3).

In the present study our metabolism measurements made at a chamber temperature of 20°C. show beyond doubt that after 10 to 13 days spent in brooding eggs the male dove loses about 10 per cent of his normal basal rate of heat production; but for female doves our data indicate no change whatever as a result of incubation. Somewhat fewer measurements made on other but similar doves at a chamber temperature of 30°C.—i.e., at or near the "critical temperature"—not only fail to show this depressing effect of incubation upon the metabolism of the male, but indicate an increase of 6 per cent. This definite difference in result, depending upon whether 20° or 30°C. is used during measurement, strongly raises the important question of the suitability and use, at least with birds, of the "critical temperature" in metabolism measurements whose purpose it is to disclose the influence of one or another factor on the current basal metabolism.

Extremely little is known concerning heat production in brooding birds.

Gerhartz (4) obtained meager and inconclusive results concerning metabolism in a brooding hen. Groebbels (5) made three measurements (with only 1 control measurement) on one pigeon (at 16°C.) under non-basal conditions and reported an increased O_2 consumption of about 30 per cent in the bird "when taken off eggs." Simpson (6) found that sitting and broody birds do not have high temperatures. Benedict, Fox and Coropatchinsky (7), (8) showed that in the incubating python the skin temperature rises 3° to 4°C. above the surrounding air.

MATERIAL AND TECHNIQUE. All measurements were made with multiple chamber apparatus, at night, in fully darkened chambers, and with all the technique earlier found (Benedict and Riddle, 9) necessary to the accurate measurement of basal heat production in these animals. Only in measurements (half of total) made on incubating birds was the following variation from that technique necessary: Since it was desirable to take the brooding birds (at night) directly from their cage and nest to the metabolism chamber, the preliminary 24-hour fast was enforced upon the birds while in their own cage instead of transferring them to a large glass cage kept at constant temperature. However, in order that the two measurements made on each bird (while incubating and while not incubating) should follow a 24-hour period at a nearly equivalent external temperature, we made 20°C. measurements of the incubating bird only on such days (spring and early summer) as afforded a mean temperature of approximately 20°; and 30° measurements on incubating birds were made only on such hot days (summer) as provided the best approach to this temperature. For the 4 tabulated groups, and for both incubation and non-incubation stages within these groups, an average respiratory quotient of 0.72 to 0.73 was obtained.

Possible changes in metabolism due to incubation may be expected to be more marked at or near the end of the term of incubation (14 days), and all measures on incubating birds were made between the tenth and fourteenth days of this period. Special observation of birds accepted for measurement during incubation made it certain that neither bird had "deserted" its nest and ceased brooding at the time of measurement. The same birds were again measured at a period which we hoped would prove to be the normal or "resting" phase of the reproductive cycle. This could be satisfactorily accomplished in the case of all males; but about one-third of the females used proved to be within 50 hours of ovulation—a condition accompanied by enlarged, actively motile oviducts, and by several blood and endocrine changes earlier described by Riddle (1), (2). The birds used belong to several different "endocrine races," or to hybrids of such races. The members of a pair, however, were usually brother and sister, or of the same inbred race; in a few cases the members of the pair were of different race. In a previous paper (10) we have shown that different races,

TABLE 1

The effect of brooding (incubation) on the basal metabolism of ring doves

DATE OF TWO MEASUREMENTS		AGE OF BIRD	HEAT PRODUCTION VALUES FOUND				
			During incubation (A)			Not incubating (B)	
A	B		Time brooding	Body weight (at end of fast)	Calories per kilo per hour	Calories per kilo per hour	Body weight (at end of fast)
			Measures made at 20°C.				
			Males				
		months	*days*	*grams*			
Av. (18)		*19.3*		*153*	*5.87*	*6.53*	*142*
			Females				
5/20–6/15		24.3	13	168	5.93	5.81	147
6/24–6/10		23.0	11	153	7.15	7.13	146
6/29–6/14		12.5	12	143	4.89	6.30*	141
5/22–7/5		24.3	11	145	5.33	5.85*	136
5/24–7/25		23.9	13	167	6.94	6.55	150
5/25–6/19		11.1	12	161	6.94	6.93	156
5/22–6/14		32.3	13	184	6.11	6.15*	165
7/1 –4/25		12.2	10	150	6.03	5.45	137
5/24–6/14		11.4	13	156	5.55	6.40*	153
5/18–4/3		9.7	13	165	6.62	6.16	141
6/20–7/18		24.1	12	146	6.36	6.87	134
7/1 –5/1		12.2	13	145	5.12	5.32	151
9/14–6/15		24.0	10	147	5.88	6.56*	146
5/25–4/27		10.7	13	137	7.60	8.31	126
6/10–7/7		12.3	12	142	6.24	6.85	134
7/1 –6/16		12.9	10	139	6.33	6.58*	138
5/24–3/18		13.7	13	172	6.12	5.80	158
6/27–6/7		11.7	12	137	6.74	6.77	124
7/25–7/5		23.9	12	158	6.41	6.54	142
Av. (19)		*17.6*		*153*	*6.23*	*6.44*	*143*
			Measures made at 30°C.				
			Males				
Av. (13)		*18.6*		*150*	*4.77*	*4.49*	*141*
			Females				
Av. (13)		*19.4*		*152*	*4.74*	*4.87*	*141*

* At or near ovulation.

like the two sexes, diverge quite unequally from their 20° heat production values when they are measured at 30°C.

EXPERIMENTAL RESULTS. The data are given, with the maximum of condensation, in table 1. Sixteen of the 18 males measured at 20° during incubation showed a decrease from their own non-incubation rate of heat production—the loss for all averaging 10 per cent. On the other hand, 12 of the 13 males measured at 30° show an increased heat production during incubation—the average gain for all being 6 per cent; likewise the single male common pigeon thus tested at 30° showed an increased heat production of 20 per cent. Plainly, the measurements made on males at the two temperatures give irreconcilable answers to the question, What is the effect of brooding on the basal metabolism of the male dove? Here one or the other of the temperatures used entirely conceals, even reverses, the true effect of incubation on the rate of heat production.

The measurements made on females in the "non-incubating" stage require an explanatory word since two different phases—"resting-stage" and "ovulation-stage"—of the reproductive cycle are represented in the tabulated data. The few measurements made at or near ovulation (indicated by *) probably gave higher values (5 to 10 per cent) than resting-stage values, and these should be converted into resting-stage values if they are to be added to the other measurements actually made during the resting stage. Since this correction can not be made with complete accuracy at present we note as follows: The 6 females measured at 20° at the ovulation stage produced 11 per cent more heat at that stage (6.31 Calories) than at the incubation stage (5.68 Calories); the 13 females of this group measured at the resting-stage produced exactly the same amount of heat (6.48 Calories) in this resting-stage as in the incubation-stage. Our data thus indicate that during incubation the basal metabolism of females measured at 20° suffers no change from the resting-stage value. Again, the 5 females measured at 30° in the ovulation-stage gave an average rate of heat production 3 per cent higher than that obtained while brooding eggs; the other 8 females measured in the resting-stage gave an average value 2 per cent above that obtained in the brooding period. These several calculations are not presented in the table (which gives general averages only) but they are verifiable from the data of the table. In the case of females measured at 30°C. it is clear that we find little or no difference in metabolic rate at resting-stage and incubation stage.

The data concerning difference in body weight of these adult birds at the two phases of the reproductive cycle are notably accurate and significant because every weight was obtained on a bird that had fasted for approximately 30 hours and had taken no water within the last 10 to 12 hours. For the four groups the weights obtained near the end of the brooding period show an average gain of 9 to 11 grams per bird over their weight in the

resting-stage of the cycle. Thirty of 31 males and 30 of 32 females increased their body weight at this period. Though several of these weights were obtained 3 to 4 days before the end of the brooding period the average amount of weight increase found is 7.2 per cent for the males and 7.5 per cent for the females. This change in body weight can not be considered sufficient to require the calculation of the present results in terms of surface area.

COMMENT. That the brooding of eggs is accompanied by a greater effect upon the metabolism of the male dove than upon his female mate is suggested by such general considerations as *a*, the fact that this brooding more markedly affects the amount of physical activity of the male; and perhaps *b*, by the report of Champy and Colle (11) that the testes of male pigeons undergo profound diminution of size during incubation. Observations made on our bird colony make it practically certain that the ovaries of incubating doves and pigeons do not undergo an equivalent involution.

Chief interest here centers upon the divergent answers obtained at 20° and at 30° as to the effect of incubation on the metabolism—particularly that of the male. Which of the two answers obtained has the greater probability of being true? From one point of view the metabolism of these animals is temporarily so *depressed* by a temperature of 30° alone that other factors than temperature are virtually erased by its use. On another view the production of heat is so *stimulated* by an external temperature of 20° that other factors are or may be obscured by its use. In choosing between these views one notes that if 20°C. is a stimulating temperature to doves and pigeons it (and still lower temperatures) is nevertheless a very usual, normal and nearly continuous stimulus in the life of these animals. Also, that the relative (and quite unusual) inactivity of the male during the brooding period—the male habitually broods the eggs during daylight hours and always quietly perches at night—would be expected to lead to a reduced heat production (9, and unpublished data; also work of others on animals made inactive by confinement); and a result in accord with this expectation is obtained only in measurements made at 20°. Again, the cyclically occurring increase in body weight observed in these animals during incubation is an occurrence which seems more consonant with a sparing of energy production than with an increased burning power in the animal's tissues, since the data of Riddle and Braucher show cases of increasing body weight accompanied by normal volume of food intake at this period.

SUMMARY

The basal metabolism of incubating ring doves has been measured at 20° and 30°C., and at a normal or "resting-stage" of the reproductive cycle for comparison. These birds were heavier by 7 per cent at the brooding stage of the cycle.

Seventy-four measurements made at 20°C. indicate that the basal heat production of male ring doves is decreased by 10 per cent from a normal resting-stage value after brooding eggs for 10 to 13 days, and that no change occurs in the metabolism of their female mates.

Fifty-two similar measurements made at the so-called "zone of thermal neutrality," 30°C., indicate that the basal heat production of brooding male ring doves is increased by 6 per cent and that of their female mates is not measurably changed.

Some evidence is cited in favor of the view that the results of the 20° measurements represent the true effect of the incubation factor. The results obtained in this study question the suitability of metabolism measurements made at the "zone of thermal neutrality" when the object of measurement is to disclose the influence of one or another factor on the current basal metabolism.

REFERENCES

(1) Riddle, O. Amer. Philos. Soc., 1927, lxvi, 497.
(2) Riddle, O. Endocrinol., 1929, xiii, 311.
(3) Riddle, O. and P. F. Braucher. This Journal (in press).
(4) Gerhartz, H. Pflüger's Arch., 1914, clvi, 1.
(5) Groebbels, F. Pflüger's Arch., 1927, ccxviii, 98.
(6) Simpson, S. Proc. Roy. Soc. Edinburgh, 1911–12, xxxii (Pt. 1), no. 5, 25; no. 11, 131.
(7) Benedict, F. G., E. L. Fox and V. Coropatchinsky. Proc. Nat. Acad. Sci., 1932, xviii, 209.
(8) Benedict, F. G. Carnegie Inst. Wash. Pub. no. 425, 1932, pp. 86 et seq.
(9) Benedict, F. G. and O. Riddle. Journ. Nutr., 1929, i, 497.
(10) Riddle, O., G. Christman and F. G. Benedict. This Journal, 1930, xcv, 111.
(11) Champy, C. and P. Colle. C. R. Soc. Biol., 1919, lxxxii, 818.

THE EFFECT OF FEEDING DESICCATED THYROID GLAND ON THE FLOW OF BLOOD IN THE FEMORAL ARTERY OF THE DOG

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Received for publication May 24, 1933

H. S. Plummer, in observing patients with exophthalmic goiter, years ago made the deduction that hyperthyroidism is accompanied by widespread dilatation of the peripheral blood vessels. The development of a method of measuring flow of blood in the normal intact animal made the experimental investigations of this problem possible. The following investigation was undertaken to determine whether experimentally induced hyperthyroidism in dogs is accompanied by an increased flow of blood to the hind limb, and if so to quantitate the degree of increase in relation to the normal flow.

TABLE 1

Changes in flow of blood in femoral artery following administration of desiccated thyroid gland

DOG	WEIGHT	FLOW OF BLOOD BEFORE THYROID FEEDING	FLOW OF BLOOD AFTER THYROID FEEDING	PERCENTAGE INCREASE
	kgm.	*cc. per minute*	*cc. per minute*	
1	17.3	92	270	293
2	9.0	35	102	291
3	14.4	113	370	327
4	19.8	140	370	264
5	16.8	119	260	218
6	10.0	83	170	205

As in previous experiments the thermostromuhr method of Rein as modified by Herrick and Baldes was used. The diathermy thermo-element was applied to the artery under local anesthesia. The experiments reported here were carried out on dogs as follows: 1, the animals were trained to lie quietly for several hours; 2, control observations were made on the flow of blood in the femoral artery of one limb; 3, after making the initial observation the animal was fed daily graduated doses of desiccated thyroid gland, and 4, when a 30 to 40 per cent increase in pulse rate had been pro-

duced, the second series of observations were made on the same artery as had been used previously.

Observations were made on a series of six dogs of several breeds and of varying ages, and in every instance a tremendous increase (from 200 to 300 per cent) in flow of blood in the femoral artery occurred after the administration of the thyroid gland (table 1).

SUMMARY

When the characteristic physiologic action of graduated doses of desiccated thyroid gland has been produced the flow of blood in the femoral artery of the dog, as measured by the thermostromuhr method of Rein, is increased from 200 to 300 per cent.

REFERENCES

(1) Herrick, J. F. and E. J. Baldes. Physics, 1931, i, 407.
(2) Plummer, H. S. Personal communication to the authors.
(3) Rein, H. Zeitschr. f. Biol., 1928, lxxxvii, 394.

THE CHEMICAL COMPOSITION OF SALIVA AND BLOOD SERUM OF CHILDREN IN RELATION TO DENTAL CARIES[1]

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Received for publication May 31, 1933

As a part of a group study of the cause of dental caries, thirty-two children, nine to sixteen years of age, were selected for a comparison of the dental condition, the bacteriology of the saliva and the chemistry of saliva and blood. These children, fifteen free of caries, and in most cases with no fillings, and seventeen with active caries, were chosen after a careful dental examination, to ensure having two groups of typical cases.

Saliva contains about 0.5 per cent of solid matter. Many of the inorganic constituents of this small amount have been considered important for the health of enamel. In reviewing the literature it has been difficult to find a basis for comparison of the results obtained by different workers. Variations in method of collecting the saliva, whether "resting" or "stimulated," together with the use of any one of many procedures for analysis make it almost impossible to assemble a clear picture of salivary composition. However, there seems to be a rather general agreement that variations in salivary composition between different individuals and for the same individual on different days and at different hours of the same day are sufficiently great to offset somewhat the values of salivary analyses (2, 3, 4, 7, 10, 14). This is certainly true. However, it is quite evident, in considering the literature, that too few of the various salivary constituents have been determined simultaneously or repeated on the same individual over long periods of time. Karshan, Krasnow and Krejci (8) have made the most complete comparison of saliva and blood in connection with the study of dental caries. They worked with dental students and found slight variations between the salivas of those who were free from caries and those who were known to have active caries. The variations for the constituents which they studied were not considered of sufficient magnitude to be significant in the etiology of dental caries.

Comparatively few reports have been published which deal with the

[1] This work represents part of a group study of the cause of dental caries which has been conducted in the school of Dentistry of the University of Michigan on a grant from the Children's Fund of Michigan.

composition of saliva and blood of children during the years when they are most susceptible to caries. Roskin (13) compared salivary calcium, phosphorus, chloride and pH of thirteen non-diabetic children who had soft teeth and marked caries with the same constituents of the salivas of fifteen children with resistant teeth. In this second group caries had been arrested during dietary control of diabetes. There were no demonstrable differences between the two groups and the author felt that the arrest of caries which was noted in the case of the diabetic children could not be due primarily to differences in the mineral content of their salivas. McIntosh, James and Lazarus-Barlow (11) found no difference between the pH of saliva of caries-free children and those with active caries.

Our observations have extended over a period of eighteen months and have included the following determinations: for saliva, volume, total solids, ash, calcium, phosphorus, chloride, diastatic activity, carbon dioxide capacity, titratable alkalinity and pH; for blood serum, calcium, inorganic acid-soluble phosphorus, carbon dioxide capacity and pH.

The children were brought to the laboratory at seven in the morning. After a half-hour of rest lying on a couch a sample of saliva was collected, with paraffin stimulation. Twenty-five to thirty cubic centimeters were obtained in two portions. The first was collected under oil and was used for the determination of pH and carbon dioxide capacity, while the second portion was used for all other determinations. A sample of blood was then taken from an arm vein. The blood was aspirated and collected under oil. Blood serum was used for all determinations. Three hours after breakfast second samples of saliva and blood were taken. Previous work had indicated that for saliva the values obtained on samples taken immediately preceding the two meals were sufficiently close to be compared. During the eighteen months blood samples have been obtained on two days for each child, and saliva on three days, with intervals of six months. Thus we have analyses of four blood and six saliva samples for each child.

METHODS OF ANALYSIS. *Saliva.* Two 10 cc. portions were transferred to 40 cc. platinum dishes and dried over night in an electric oven to determine total solids. This dried material was ashed in an electric muffle at a temperature just below dull red heat. The weighed ash was dissolved in three 1 cc. portions of hot hydrochloric acid (1:3) and made up to volume in a 25 cc. volumetric flask. This solution was used for the determination of calcium by the Clark and Collip (1) modification of the Kramer and Tisdall (9) method, and for phosphorus by the procedure of Fiske and Subbarrow (5). The other constituents were determined on fresh saliva: chloride by the method of Van Slyke (15), carbon dioxide capacity by that of Van Slyke and Cullen (16),[2] using 1 cc. samples, and diastase according to the procedure of Myers and Dellenbaugh (12). In reporting results for

[2] The determinations of carbon dioxide capacity were made by Elise Morrell.

diastase, figures are given for the starch digested without the addition of sodium chloride to activate the enzyme. Titratable alkalinity was determined by titrating 5 cc. portions of saliva with 0.02 N HCl, using methyl orange as the indicator. In the early work, pH was determined with the quinhydrone electrode, and later, colorimetrically in a 1:10 dilution.

Blood serum. The Clark and Collip (1) modification of the Kramer and Tisdall (9) procedure was used for calcium and for inorganic acid-soluble phosphorus, the method of Fisk and Subbarrow (5). Carbon dioxide capacity was determined by the usual Van Slyke and Cullen method (16) and pH with the quinhydrone electrode.

Dental examinations[3] were made at the beginning and at the end of, and once during the eighteen months. During this time there were few decided changes in the clinical picture. Of those who were originally classed as caries free, one developed a cavity (O.L) and four were reported as questionable at the last examination (C.H., J. F., L. B., E. E.). Three of those originally classed as having active caries have shown no evidence of activity during the period of study and are now considered questionable (W. B., D. I., D. W.). On the whole we have not felt justified in changing the original classifications because of the slight changes in the dental findings.

Bacteriological studies of saliva were made on the same days on which blood and saliva were collected for chemical analysis. The full report of this survey will be published separately. Bacillus acidophilus was checked quantitatively and was found to be consistently low or negative in the salivas of those children who were free of caries. For those with active caries, the count was moderate or high.[4]

Table 1 and table 2 summarize the results of the chemical studies of both saliva and serum for the members of two groups, while table 3 shows the range of values for the caries-free children compared with those who had active caries. For blood serum there is no definite level of calcium, inorganic acid-soluble phosphorus, carbon dioxide capacity or pH which is characteristic of either group. For most of the constituents of saliva the same is true. The variations are greater between individuals in the same group than they are between the groups as a whole. These findings are in harmony with those of other workers for the saliva and blood serum of adults. The results for calcium and phosphorus of saliva also agree well with those which have been given in an earlier report from this laboratory (7).

However, for carbon dioxide capacity and for titratable alkalinity of

[3] The dental examinations were made by Dr. Russell W. Bunting, Dr. Dorothy G. Hard and Dr. Philip Jay.

[4] Personal communication, from unpublished work of Faith P. Hadley.

TABLE 1

Average analyses of saliva and blood serum of caries-free children

SUBJECT	AGE*		SALIVA										BLOOD SERUM			
			Volume	Solids	Ash	Calcium	Phosphorus	Chloride	Diastase†	Titratable alkalinity‡	Carbon dioxide capacity§	pH	Calcium	Phosphorus	Carbon dioxide capacity§	pH
	yr.	*mo.*	*cc./min.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*					*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
J. E.	9	0	0.6	555	205	4.9	16.3	35.4	24.5	115.0	25.5	7.4	10.9	5.6	50.2	7.34
C. H.	9	6	1.4	489	264	5.5	12.2	49.5	12.2	133.2	41.2	7.4	10.2	5.0	48.7	7.34
P. B.	9	7	0.9	486	193	6.1	16.6	47.7	11.1	118.0	26.9	7.6	10.4	4.3	51.6	7.31
E. B.	10	6	1.4	587	228	5.6	17.8	50.7	38.2	106.4	25.9	7.2	9.9	5.7	52.0	7.33
W. G.	10	6	1.6	425	210	6.5	13.6	41.6	12.3	102.0	35.9	7.4	10.4	4.1	54.7	7.30
O. L.	10	8	1.2	522	192	5.2	16.0	47.4	23.7	117.5	32.4	7.3	10.4	4.7	52.9	7.36
R. P.	11	2	1.6	557	231	6.1	15.8	31.2	18.1	146.8	45.9	7.4	10.5	4.4	53.2	7.36
J. F.	11	6	1.5	444	208	4.8	11.4	39.5	17.6	122.3	39.5	7.5	10.0	5.5	49.5	7.32
M. B.	11	6	1.0	544	216	5.2	15.9	33.9	12.5	108.1	26.5	7.2	10.0	4.2	53.2	7.39
J. E.	11	10	0.8	626	228	6.1	16.1	53.7	45.1	115.1	30.1	7.2	10.2	5.3	52.1	7.33
V. D.	13	2	1.0	431	166	5.6	12.9	53.6	41.0	111.0	27.8	7.2	10.4	4.8	51.9	7.35
L. B.	13	8	1.3	542	209	5.0	15.6	42.6	13.1	115.4	32.1	7.1	10.0	4.9	57.8	7.43
M. S.	13	10	1.3	562	201	4.9	14.5	62.7	45.2	94.7	20.1	6.9	10.3	5.5	53.8	7.30
M. R.	14	0	1.3	476	224	4.5	12.8	37.2	18.0	110.3	33.7	7.6	10.4	4.6	54.9	7.40
E. E.	15	8	1.3	495	183	4.4	13.3	46.9	27.4	94.8	21.6	7.4	10.2	4.3	54.9	7.33
Average	11	8	1.2	516	210	5.4	14.7	44.9	24.0	114.0	31.0	7.3	10.3	4.9	52.8	7.34

* Age at first examination.

† Per cent starch hydrolyzed under standard conditions.

‡ Cubic centimeter 0.02 N HCl per 100 cc. saliva.

§ Cubic centimeter per 100 cc. saliva or serum.

TABLE 2

Average analyses of saliva and blood serum of caries-susceptible children

SUBJECT	AGE*		SALIVA										BLOOD SERUM			
			Volume	Solids	Ash	Calcium	Phosphorus	Chloride	Diastase†	Titratable alkalinity‡	Carbon dioxide capacity§	pH	Calcium	Phosphorus	Carbon dioxide capacity†	pH
	yr.	*mo.*	*cc./min.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*					*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
W. B.	8	9	0.7	486	196	5.8	16.0	33.7	12.8	105.2	25.7	7.3	10.2	4.5	50.6	7.36
D. I.	8	9	0.9	526	201	6.6	13.6	55.8	25.6	107.2	28.9	7.4	10.3	5.3	53.9	7.35
D. W.	8	9	1.3	414	181	4.9	12.2	40.4	32.0	94.5	20.6	7.3	10.1	5.6	50.0	7.32
H. B.	10	0	0.6	612	212	5.7	13.9	39.3	20.2		16.6	7.2	10.4	5.9	53.5	7.36
C. B.	10	3	0.9	415	191	5.0	11.3	40.3	21.3		20.7	7.1	10.5	5.8	53.3	7.35
A. E.	10	9	1.4	528	248	5.3	16.0	41.3	18.3	137.2	50.9	7.3	9.7	4.9	56.7	7.35
A. W.	10	9	1.5	429	168	5.4	16.0	38.3	45.5	96.5	24.7	7.2	10.2	5.6	47.8	7.33
J. R.	12	0	0.9	528	184	4.8	16.9	31.2	23.1	106.4	30.6	7.4	10.2	5.2	53.2	7.38
W. W.	12	0	0.6	543	210	5.3	16.9	53.2	36.1	75.0	13.5	6.9	10.1	4.3	56.6	7.33
L. F.	13	0	1.5	459	197	5.3	12.8	31.3	25.2	84.2	18.7	7.1	9.8	4.5	53.3	7.33
R. L.	13	2	0.8	527	172	4.1	13.0	46.8	41.2	77.4	18.0	7.0				
P. S.	13	5	1.6	500	204	6.3	16.9	33.7	15.0	90.2	20.9	7.2	10.1	5.2	52.8	7.33
F. B.	13	7	1.0	429	185	5.7	11.5	32.1	13.1	93.0	24.8	7.1	9.8	5.3	52.2	7.32
M. W.	13	9	0.8	490	210	4.4	12.4	49.3	38.7	69.0	13.1	7.1	10.4	4.7	53.3	7.34
N. I.	15	0	0.9	546	204	5.7	14.6	47.2	29.1	74.0	11.4	6.8	9.9	5.5	55.3	7.34
J. D.	15	0	1.0	678	218	4.6	15.1	39.9	41.0	89.2	8.3	6.8	10.3	3.4	54.5	7.32
I. H.	16	5	2.4	393	165	4.6	10.5	41.2	31.2	79.4	19.5	7.2	10.0	3.9	51.6	7.33
Average.	12	0	1.1	500	197	5.3	14.1	40.9	27.6	91.9	21.6	7.1	10.1	5.0	53.0	7.34

* Age at first examination.

† Per cent starch hydrolyzed under standard conditions.

‡ Cubic centimeter 0.02 N HCl per 100 cc. of saliva.

§ Cubic centimeter per 100 cc. of saliva or serum.

saliva there seem to be real differences. The average carbon dioxide capacity for the caries-free children is 31.0 cc. per 100 cc. of saliva, while for those with active caries it is 21.6. With the exception of A. E. (table 2), all the values for carbon dioxide capacity for the children with active caries are below the average of those who are free from caries. In the case of titratable alkalinity the average for those free from caries is 114.0 cc. of 0.02 N HCl per 100 cc. of saliva, in contrast to 91.9 for the members of

TABLE 3

Summary of analyses of saliva and blood serum of caries-free and caries-susceptible children

Expressed in amounts per 100 cc. of saliva or serum

	CARIES-FREE		CARIES-SUSCEPTIBLE	
	Range	Average	Range	Average
Saliva:				
Volume*	0.6– 1.6	1.2	0.6– 2.4	1.1
Solids, mgm	425–626	516	393–678	500
Ash, mgm	166–264	210	165–248	197
Calcium, mgm	4.4– 6.5	5.4	4.1– 6.6	5.3
Phosphorus, mgm	11.4– 17.8	14.7	9.1– 16.9	14.1
Chloride, mgm	31.2– 62.7	44.9	31.2– 55.8	40.9
Diastase†	11.1– 45.2	24.0	12.8– 45.5	27.6
Titratable alkalinity‡	94.7–146.8	114.0	69.0–137.2§	91.9
Carbon dioxide capacity, cc	20.1– 45.9	31.0	8.3– 50.9§	21.6
pH	6.9– 7.6	7.3	6.8– 7.4	7.1
Blood serum:				
Calcium, mgm	9.9– 10.9	10.3	9.7– 10.4	10.1
Phosphorus, mgm	4.1– 5.7	4.9	3.4– 5.9	5.0
Carbon dioxide capacity, cc	48.7– 57.8	52.8	47.8– 56.7	53.0
pH	7.30– 7.43	7.34	7.32– 7.38	7.34

* Cubic centimeter per minute.

† Per cent starch hydrolyzed under standard conditions.

‡ Cubic centimeter 0.02 N HCl per 100 cc. of saliva.

§ Figures of this magnitude were found for one individual in this group. All others were below the average for the caries-free group.

the other group. Again, subject A.E. is the only one with a figure above the average for the caries-free group.

If the saliva, because of its acid neutralizing power as shown by carbon dioxide capacity and titratable alkalinity, has any effect on caries prevention, we might expect to find definite chemical changes in the salivas of those subjects who are now reported as questionable, or in the one case where caries has developed during the time the children have been under observation. A study of the results for these individuals does not give a

clear cut picture. Of the four caries-free children, whose dental condition is now questioned, there is but one, E. E., whose saliva shows a carbon dioxide capacity or titratable alkalinity on a level with those subjects who showed marked caries throughout the entire period of observation. In the case of the three with a decreased tendency to caries, the salivas of two, W. B. and D. I., show analyses which compare favorably with those of the caries-free group, as far as these two constituents are concerned. Since so few subjects have shown a change in susceptibility to caries, no conclusions can be drawn concerning any chemical factors which may be concerned with the change in dental findings.

SUMMARY

1. Analyses have been made of saliva and blood serum, using as subjects fifteen children who were free from caries and seventeen who had active caries. These children were from nine to sixteen years of age.

2. The carbon dioxide capacity of the saliva of the caries-free children was about 44 per cent higher than that of the saliva of children of the same age who had active caries.

3. The titratable alkalinity of the saliva of caries-free children was about 24 per cent higher than that of the children of the same age who had active caries.

4. In other respects, analyses of saliva (total solids, ash, calcium, phosphorus, chloride, diastatic activity and pH) showed no consistent differences between the two groups.

5. Blood serum calcium, inorganic acid-soluble phosphorus, carbon dioxide capacity and pH were the same for the children of both groups.

REFERENCES

(1) Clark, E. P. and J. B. Collip. 1925. Journ. Biol. Chem., lxiii, 461.
(2) Clark, G. W. and K. L. Carter. 1927. Journ. Biol. Chem., lxxiii, 391.
(3) Clark, G. W. and L. Levine. 1927. This Journal, lxxxi, 264.
(4) Clark, G. W. and J. S. Shell. 1927. Dental Cosmos, lxix, 500, 605.
(5) Fiske, C. W. and Y. Subbarow. 1925. Journ. Biol. Chem., lxvi, 375.
(6) Henderson M. and J. A. P. Millet. 1927. Journ. Biol. Chem., lxxv, 559.
(7) Hubbell, R. B. and R. W. Bunting. 1932. Journ. Nutr., v, 599.
(8) Karshan, M., F. Krasnow and L. Krejci. 1931. Journ. Dent. Res., xi, 573.
(9) Kramer, B. and F. F. Tisdall. 1921. Journ. Biol. Chem., xlvii, 475.
(10) McClelland, J. R. 1922. This Journal, lxiii, 127.
(11) McIntosh, J., W. W. James, and P. Lazarus-Barlow. 1926. Brit. Journ. Exp. Pathol., vi, 260.
(12) Myers, V. C. and A. G. Dellenbaugh. 1918. Proc. Soc. Exp. Biol. and Med., xvi, 18.
(13) Roskin, M. 1928. Proc. Soc. Exp. Biol. and Med., xxv, 465.
(14) Starr, H. E. 1922. Journ. Biol. Chem., liv, 55.
(15) Van Slyke, D. D. 1921. Journ. Biol. Chem., xlix, 487.
(16) Van Slyke, D. D. and G. E. Cullen. 1917. Journ. Biol. Chem., xxx, 289.

HEMOGLOBIN REGENERATION IN GASTRECTOMIZED DOGS

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Received for publication May 27, 1933

The almost constant finding of achlorhydria in cases of pernicious anemia is well known. Recent advances in the study of pernicious anemia indicate that the stomach may bear a definite and very significant relation to the genesis of the disease. The occurrence of achlorhydria in pernicious anemia, the supervention of the disease in humans following gastrectomy, the marked therapeutic effects of products of gastric digestion and of preparations of gastric tissue are convincing evidence of the existence of this relationship. That achlorhydria is frequently associated with an anemia secondary in type is also attracting considerable interest. A microcytic hypochromic anemia accompanied by diminution or absence of free hydrochloric acid in the gastric juice, has been described as a definite clinical entity by a number of writers including Faber (1913), Witts (1930), Hurst (1932), Meulengracht (1932), Haden (1932) and many others.

This association between gastric achylia and anemia has stimulated a number of investigators to study the effect of artificial gastric achylia produced by gastrectomy, etc., in the experimental animal. Most of these investigators (Carvallo and Pachon, 1893; Monari 1896; Grohe, 1903; Kaiser, 1878; Dagaew, 1913; Carrel, Meyer and Levene, 1910, and Mann 1929) failed to observe the occurrence of an anemia following such operations. Recently, however, Ivy, Morgan and Farrell (1931) have reported the occasional development of a spontaneous anemia in gastrectomized dogs and in dogs with a pouch of the entire stomach. They reported an increased tendency to an anemia in gastrectomized female dogs that became pregnant. This they postulated as indicating that the gastrectomy had reduced the factor of safety in the dog and that the additional strain of pregnancy was sufficient to produce an anemia.

These observations have prompted us to determine in a more or less quantitative way the effect of gastrectomy upon the hemopoietic functions of the normal dog. Hemoglobin production and the qualitative changes in the blood picture were determined in gastrectomized dogs maintained at an anemic level according to the technique employed by Whipple and his collaborators.

EXPERIMENTAL. A complete gastrectomy was performed in five healthy, adult, mongrel dogs anastomosing the esophagus to the duodenum in each case. After the animals had completely recovered from the operation, observations of the erythrocyte count, hemoglobin, blood volume, etc., were made. The dogs were then bled at intervals from the external jugular vein to approximately 50 per cent of the original total circulating hemoglobin and maintained at this level by subsequent bleedings for several weeks until hemoglobin production was relatively stable at which time the storage reserve of hemoglobin and hemoglobin precursors was presumably exhausted. They were then placed on several different diets and the amount of hemoglobin necessary to withdraw to maintain this anemic level determined. This represents the hemoglobin production during such a period except for that fraction necessary for maintenance purposes.

TABLE 1

DOG NUMBER	NUMBER OF DAYS ANEMIC PRIOR TO EXPERIMENTAL PERIOD	NUMBER OF DAYS EXPERIMENTAL PERIOD	TOTAL HEMOGLOBIN AT START	HEMOGLOBIN WITHDRAWN	TOTAL HEMOGLOBIN AT END	TOTAL HEMOGLOBIN PRODUCTION	HEMOGLOBIN PRODUCTION PER DAY
			grams	*grams*	*grams*	*grams*	*grams*
10	47	60	50.6	6.39	50.6	6.39	0.106
11	34	49	53.2	32.09	47.7	26.6	0.542
12	49	34	96.0	33.23	65.2	3.15	0.09
Average							0.246

Hemoglobin was determined colorimetrically by Newcomer's methods. Blood volume determinations were made by the method of Keith and Rowntree as modified by Whipple.

RESULTS. 1. *Hemoglobin production in gastrectomized dogs on a stock diet.* Three gastrectomized dogs were maintained at an anemic level on the stock diet of the laboratory consisting of bread, milk, bone meal, corn meal and a small proportion of a commercial dog food preparation. The essential findings are indicated in the accompanying table (table 1).

In two dogs maintained at an anemic level for 47 and 49 days respectively prior to the experimental period, the average daily hemoglobin production during the experimental period on the stock diet was approximately 0.1 gram. In one dog maintained at an anemia level for 34 days prior to the experimental period, the average daily hemoglobin production during the ensuing test period of 49 days was 0.542 gram. This high figure we believe due to the failure to exhaust sufficiently the reserve of blood forming material prior to the experimental period. The necessity of doing this has

been repeatedly pointed out by Whipple and his co-workers and has had ample confirmation during the course of our work.

2. *Hemoglobin production in gastrectomized dogs on a meat diet.* Four gastrectomized dogs were maintained at an anemic level while on a meat diet, which consisted of bread and milk ad libitum and approximately one pound of ground beef, brought to a boil for a short time. One dog was carried through three experimental periods and two of them through two so that eight observations were made. The essential results appear in the table below (table 2).

As seen from the table, the average daily hemoglobin production is approximately 0.22 gram. It should be noted that dog 14 was not maintained at an anemic level prior to the experimental period. This un-

TABLE 2

Hemoglobin production in gastrectomized dogs—meat diet

DOG NUMBER	DAYS ANEMIC PRIOR TO EXPERIMENTAL PERIOD	NUMBER OF DAYS EXPERIMENTAL PERIOD	TOTAL HEMOGLOBIN AT START	HEMOGLOBIN WITHDRAWN	TOTAL HEMOGLOBIN AT END	TOTAL HEMOGLOBIN PRODUCTION	HEMOGLOBIN PRODUCTION PER DAY
			grams	*grams*	*grams*	*grams*	*grams*
10	109	29	50.6	4.15	45.5		
10		24	61.0		69.0	8.00	0.33
10		37	71.0	15.74	64.0	8.74	0.236
11	83	30	47.7	12.4	39.1	3.8	0.127
11		37	64.0		75.0	11.0	0.298
12	104	54	96.4	19.3	75.0		
12		37	95.0	23.8	85.0	13.8	0.373
14	None	112	168.0	128.0	81.0	41.0	0.371
Average							0.217

doubtedly tends to give an erroneously high figure for this animal but as the experimental period was quite long (112 days), this tendency is minimized and the figures are not greatly different from those of the other animals.

3. *Hemoglobin production in gastrectomized dogs on a diet of meat and liver.* Three gastrectomized dogs were maintained at an anemic level while on a diet consisting of one pound of chopped raw beef liver supplemented with a small amount of bread, milk and boiled beef. The data for hemoglobin production on this diet are indicated in the following table (table 3).

As seen from the table, the average hemoglobin production on the meat-liver diet was approximately 0.32 gram per day. It may be noted that the days that these animals were maintained in an anemic state while on a

meat diet have been included in specifying the number of days anemic prior to the experimental period, because the hemoglobin production during these periods was of such a low order that it seemed unlikely any reserve store of hemoglobin or hemoglobin building materials had accumulated.

4. *Hemoglobin production in gastrectomized dogs on a meat diet supplemented with liver extract no. 343.*[1] Two gastrectomized dogs were maintained at an anemic level while on the meat diet specified above. Dog 10 received three teaspoonfuls of liver extract daily and dog 12 two tea-

TABLE 3

Hemoglobin production in gastrectomized dogs—meat and liver

DOG NUMBER	DAYS ANEMIC PRIOR TO EXPERIMENTAL PERIOD	NUMBER OF DAYS EXPERIMENTAL PERIOD	TOTAL HEMOGLOBIN AT START	HEMOGLOBIN WITHDRAWN	TOTAL HEMOGLOBIN AT END	TOTAL HEMOGLOBIN PRODUCED	HEMOGLOBIN PRODUCED PER DAY
			grams	*grams*	*grams*	*grams*	*grams*
10	184	42	69.0		71.0	2.0	0.048
11	143	57	39.1		64.0	24.9	0.437
12	158	42	75.0		95.0	20.0	0.475
Average							0.319

TABLE 4

Hemoglobin regeneration in gastrectomized dogs—meat and liver extract number 343

DOG NUMBER	DAYS ANEMIC PRIOR TO EXPERIMENTAL PERIOD	NUMBER OF DAYS EXPERIMENTAL PERIOD	TOTAL HEMOGLOBIN AT START	HEMOGLOBIN WITHDRAWN	TOTAL HEMOGLOBIN AT END	TOTAL HEMOGLOBIN PRODUCTION	HEMOGLOBIN PRODUCTION PER DAY
			grams	*grams*	*grams*	*grams*	*grams*
10	122	22	45.5		61.0	15.5	0.70
12	237	29	85.0	7.37	87.0	9.37	0.323
Average							0.51

spoonfuls in addition. The essential results appear in the accompanying table (table 4).

5. *Hemoglobin production in gastrectomized dogs on a meat diet supplemented with liver extract 55 (Lilly).*[2] Two gastrectomized dogs were maintained at an anemic level while on the meat diet specified above. In addi-

[1] We are indebted to Eli Lilly & Co. for the liver extract used in these experiments.

[2] We are indebted to Eli Lilly & Co. for the liver extract used in these experiments.

tion, each dog received two teaspoonfuls daily of liver extract no. 55. The essential results appear in the accompanying table (table 5).

SUMMARY. The data with respect to hemoglobin production in gastrectomized dogs may be summarized as follows: When maintained at an anemic level on the laboratory stock diet, there is an average production of from 0.1 to 0.246 gram of hemoglobin per day or of 1.4 to 3.4 grams per 14 day period; on a meat diet (beef muscle) of 0.217 gram per day or 3.04 grams per 14 day period; on a meat and liver diet 0.319 gram per day or 4.47 grams per 14 day period; on meat plus liver extract no. 343 of 0.51 gram per day or 7.11 grams per 14 day period; and on a meat plus liver extract no. 55 of 1.61 grams per day or 22.54 grams per 14 day period. All of our diets are not completely identical to those employed by Whipple and his co-workers in determining the hemoglobin regeneration capacity of normal dogs, but they are sufficiently comparable to draw a number of conclusions with respect to the effect of gastrectomy on hemoglobin regeneration. The diets in our experiments which are least comparable to those employed by Whipple are the stock diet and the meat diet. On these diets, normal dogs in our laboratory have been found to regenerate 21.7 and 29.12 grams of hemoglobin respectively per 14 day period. The gastrectomized dogs thus have little more than ten per cent of this capacity. More striking still is the relative lack of response of the gastrectomized dog to liver feeding. Whipple et al. have found normal dogs to produce an average of 95 grams of hemoglobin per 14 day period whereas the gastrectomized dogs show but a slight increase from that on a meat diet (3.038 grams per 14 day period to 4.466 grams). Similarly the gastrectomized dogs show but a negligible increase as a result of administering liver extract no. 343. The only striking increase in hemoglobin regeneration in the gastrectomized dogs was seen in the case of liver extract no. 55. On a meat diet alone, hemoglobin production was 3.038 grams per 14 day period, while when liver extract no. 55 was added, the regeneration was

TABLE 5

Hemoglobin regeneration in gastrectomized dogs, meat and liver extract number 55

DOG NUMBER	DAYS ANEMIC PRIOR TO EXPERIMENTAL PERIOD	NUMBER OF DAYS EXPERIMENTAL PERIOD	TOTAL HEMOGLOBIN AT START	HEMOGLOBIN WITHDRAWN	TOTAL HEMOGLOBIN AT END	TOTAL HEMOGLOBIN PRODUCTION	HEMOGLOBIN PRODUCTION PER DAY
			grams	*grams*	*grams*	*grams*	*grams*
10	263	33	64.0	31.7	89.2	56.9	1.725
14	132	29	81.0	32.0	92.5	43.5	1.5
Average							1.61

22.54 grams in a similar period. As this extract contains added iron in the form of iron ammonium citrate and as whole liver alone was practically ineffective in the gastrectomized dogs, present studies are under way to determine the response to iron compounds alone.

One gastrectomized dog (not included in any of the above experiments) developed a spontaneous anemia while kept on the laboratory stock diet and died 80 days after the operation from a terminal pneumonia. The total hemoglobin fell from 106 grams to 48 grams and the erythrocyte count from 6.6 million per cubic millimeter to 4.8 million.

Discussion. At the beginning of these experiments, in addition to the objectives previously stated, the questions naturally arose as to whether the gastrectomized dog maintained at an anemic level for long periods of time might not either develop an anemia similar in character to pernicious anemia or, as a more practical matter, might not respond sufficiently characteristically to the liver preparations effective in pernicious anemia that a method of standardizing such preparations without resort to the clinic could be developed. The data here reported show no indications of the development of an anemia of this type. Even after several months at an anemic level, there is a relative microcytosis, low color index, low volume index, and the stained smear does not show the characteristic morphological picture of pernicious anemia. Also the observations reported here indicate that the gastrectomized dog responds only feebly to either whole liver or liver extract no. 343. The favorable response to liver extract no. 55 may possibly be attributed to the iron content (although this remains to be determined). In this respect also the anemia is obviously different from that of the pernicious type. It would seem either that the failure of the gastrectomized dog to develop a pernicious anemia indicates that he is biologically different from man in this respect, or that in man as well as in the dog, achylia or gastrectomy, as the case may be, is only one of several inter-related factors in the etiology of this disease. That the gastrectomized dog has a markedly reduced hemoglobin regenerating capacity would seem to favor this latter view. In harmony with this are the clinical observations that neither achylia nor gastrectomy in the human is invariably followed by pernicious anemia. On the other hand, it must be considered that the time element after the achylia or gastrectomy may be important and that in our dogs as well as those of other workers, not sufficient time has elapsed to warrant the conclusion that the gastrectomized dog may not develop a pernicious anemia.

CONCLUSIONS

1. The gastrectomized dog has a markedly reduced capacity to regenerate hemoglobin after hemorrhage.

2. The gastrectomized dog rendered anemic by repeated bleeding shows

but little augmentation in hemoglobin regeneration as a result of liver feeding or the administration of liver extract no. 343.

3. Liver extract no. 55 has a definitely beneficial effect on hemoglobin regeneration in the anemic gastrectomized dog.

4. A spontaneous anemia developed in one gastrectomized dog maintained on a stock diet.

5. Neither the spontaneous anemia in one dog nor the induced anemias in four gastrectomized dogs showed any tendency to develop the characteristic features of pernicious anemia.

REFERENCES

CARREL, A., G. M. MEYER AND P. A. LEVENE. 1910. This Journal, xxvi, 369.
CARVALLO, J. AND V. PACHON. 1893. Compt. rend. Soc. de Biol., xlv, 929.
KAISER, F. F. 1878. Zeitschr. z. Operat. chir., 141.
DAGAEW, W. F. 1913. Mitt. a. d. Grenzgeb., xxvi, 176.
FABER, K. 1913. Berl. klin. Wochenschr., l, 958.
GROHE, B. 1903. Arch. f. exper. Path. u. Pharm., xlix, 114.
HADEN, R. L. 1932. Journ. Amer. Med. Assn., xcix, 1398.
HURST, A. F. 1932. Quart. Journ. Med., i, 157.
IVY, A. C., J. E. MORGAN AND J. I. FARRELL. 1931. Surg., Gynec. and Obst., liii, 611.
MANN, F. C. 1929. Proc. Staff Meet. Mayo Clinic, iv, 293.
MEULENGRACHT, E. 1932. Acta Med. Scand., lxxviii, 387.
MONARI, U. 1896. Beitr. f. klin. Chir., xvi, 479.
WITTS, L. J. 1930. Guy's Hosp. Repts., lxxx, 253.

ELECTROMYOGRAPHIC STUDIES OF THE GASTRO-INTESTINAL TRACT

V. Further Inquiries into the Origin of Potential Variations of the Small Intestine by Means of Certain Drugs[1]

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Received for publication May 27, 1933

Previously it has been reported that the regular, characteristic variations of potential, which can be registered from excised segments of the small intestine, can be altered in relation to the recorded mechanical movements by means of epinephrine and nicotine. Epinephrine inhibits the recorded muscular movements, but leaves the recorded rhythmic potential variations relatively unaffected. Nicotine abolishes the characteristic alterations of potential, while the rhythmic contractual movements are undiminished or increased. This and other evidence was taken to indicate that the observed electrical changes are not true action currents of muscle, and the hypothesis was ventured that they had their origin in a rhythmic activity of the intrinsic nervous plexus.

Observations made with the use of other drugs accord with this hypothesis. Here are reported results obtained with atropine, ephedrine, and curare. The method of experiment is identical with that described in previous publications. An excised strip of the small intestine of a rabbit was used, immersed in a bath containing 100 cc. of physiologic saline solution. A simultaneous mechanical and potential record was made. The drugs employed were injected into the bath.

The effects obtained with atropine were similar to those previously reported for epinephrine. The recorded mechanical movement was completely arrested when atropine was applied (1 cc., 1:1000); the electrical variations continued (fig. 1, *b*). This result was somewhat surprising. The ganglia and nerves of Auerbach's plexus are generally considered part of the parasympathetic nervous system of the intestine. Since the effect of nicotine on the electrical alterations was considered to be due to its

[1] Presented in abstract in the Proceedings of the American Physiological Society, 1933.

[2] Work done on a grant from the Josiah Macy, Jr., Foundation under the direction of Walter C. Alvarez, Associate Professor of Medicine, The Mayo Foundation, University of Minnesota.

action on the intrinsic ganglia, it was expected that atropine which is a parasympathetic drug would also influence the potential alterations.

Neither the effect of atropine nor epinephrine was noticeably affected by the previous application of ergotoxin.

The effects obtained with ephedrine were different from those of epinephrine. It was difficult even with large doses of ephedrine to obtain com-

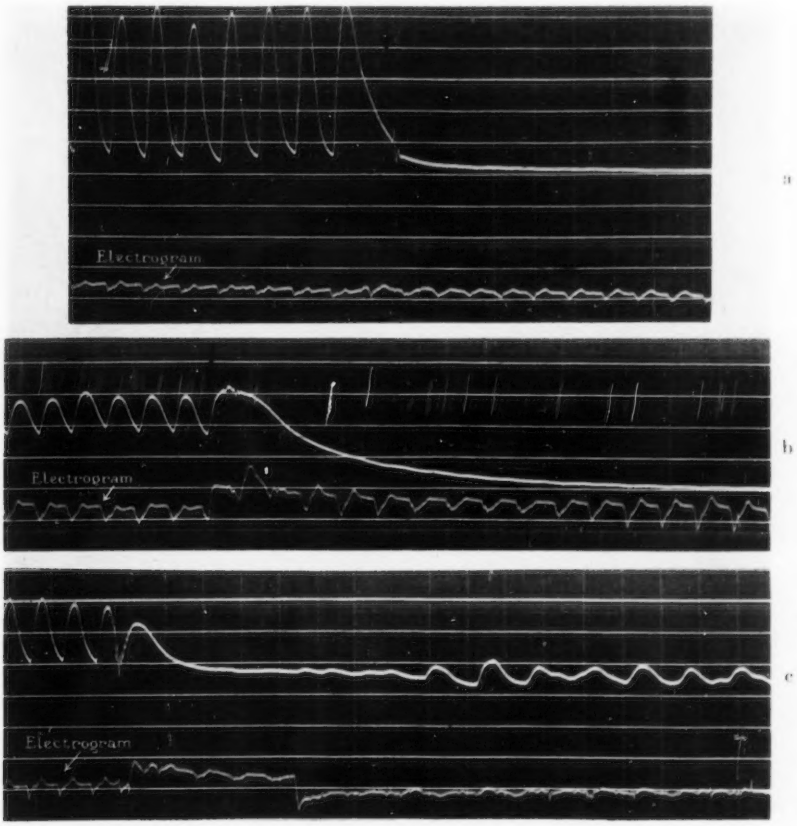

Fig. 1. *a*, The effect of epinephrine on excised intestinal strip. At black arrow, epinephrine 1 cc., 1:3000 was injected into bath containing 100 cc. physiologic saline solution. The recorded mechanical movements are arrested. The characteristic potential cycle continues. *b*, Effect similar to that shown in *a*, obtained with atropine 1 cc., 1:1000 injected at arrow. *c*, Ephedrine 1 cc., 1:10 injected at arrow. The relaxation of the contractual movements is not so complete as obtained with epinephrine, but the electrical variations are markedly disturbed.

plete relaxation of the intestine. Yet the concomitant electrical changes were notably affected, sometimes violently and always appreciably (fig. 1, *c*). From this it is argued that ephedrine affects in one manner or another the intrinsic nervous plexus, in addition to any effect it may have on the muscular tissue. The contrast between the effects of epinephrine and ephedrine affords additional evidence to what has previously been advanced, that the muscular contractions of the intestine are, to some degree at least, independent of the charactistic electrical alterations. For, if the electrical variations depended completely on the muscular contractions,

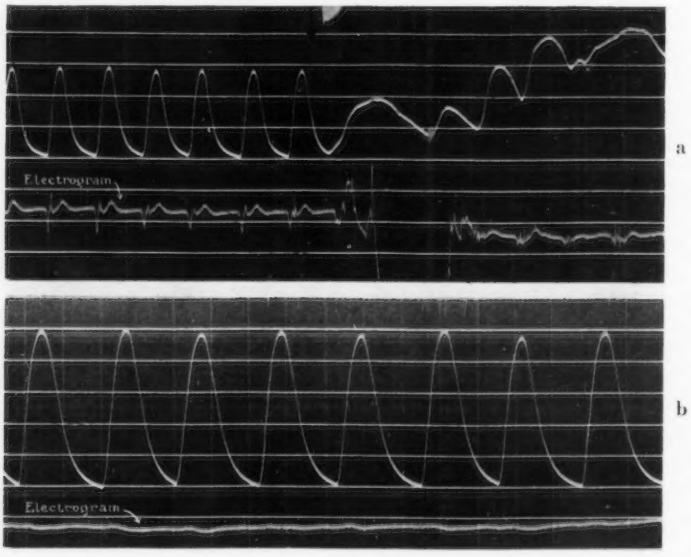

Fig. 2. *a*, Normal record, excised strip of intestine. At arrow curare 2 cc., 1 per cent injected into bath. *b*, Thirty minutes later. The contractual movements have returned to vigorous rhythm. The electrogram shows only feeble periodic oscillation.

the effects of various agents on the electrical alterations would be in proportion to the effect on the muscular contractions. But it is found that ephedrine, which is less effective than epinephrine on the mechanical contraction, is markedly more effective in disturbing the characteristic electrical alterations.

The effects produced by use of curare were similar to those previously reported for nicotine qualitatively, but differed quantitatively. A large dose of curare abolished the characteristic electrical cycles, while the mechanical contractual movement was retained (fig. 2). The initial effect of

curare was usually relaxation of the mechanical movement. The electrical alterations, which had been proceeding regularly, suffered marked erratic disturbance. These effects were transient. Very soon the rhythmic mechanical movements began to return, but the electrical variations were atypical in appearance. After about fifteen minutes the mechanical movements returned, and proceeded with very regular rhythm, large amplitude and a slow rate (6 per minute). The electrical record at this time showed only feeble oscillations, occurring periodically during contraction. The characteristic cycle of potential changes present before the application of curare was no longer evident. Gradually the mechanical movements ceased, with a progressive disappearance of the recorded electrical variations. When curare was used, therefore, essentially the same series of events was seen that followed the use of nicotine. The chief difference was that the disappearance of the electrical variations was not so complete as that which occurred after the application of nicotine, even when the dose of curare was large.

All these observations are consistent with the view that the characteristic potential cycles which have been described, originate in a physiological rhythm within the intrinsic nervous plexus. That the effect of curare is, like nicotine, to abolish these characteristic potential alterations, while the rhythmic contractions continue, means in terms of the hypothesis here advanced, that both drugs affect specifically the nervous tissue. The effect of curare on smooth muscle is not discussed in the literature, but its nicotine-like action has been frequently noted. To this degree there is agreement between the generally stated view of the pharmacological action of the drugs employed and the view here expressed. The effects of epinephrine and atropine are somewhat difficult to reconcile with the orthodox view that these drugs affect respectively the sympathetic and parasympathetic nervous systems. The effects of both drugs on the excised intestine, either as regards the electrical or mechanical record, are indistinguishable one from the other. This would appear to mean that the mechanism of the effect of both in this situation is essentially the same. That both affect the mechanical and not the electrical records, would point to an action on the muscle tissue rather than the nerve tissue. Ephedrine, on the other hand, appears to affect both.

REFERENCES

(1) Berkson, J. This Journal, 1933, civ, 67.
(2) Berkson, J. Proc. Amer. Physiol. Soc., This Journal, 1933, cv, 5.
(3) Puestow, C. B. Proc. Amer. Physiol. Soc., This Journal, 1933, cv, 81.

ELECTROMYOGRAPHIC STUDIES OF THE GASTRO-INTESTINAL TRACT

VI. Recovery of Characteristic Electropotential Variations of the Small Intestine Following Application of Nicotine, by Restoration of Circulation

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Received for publication May 27, 1933

My initial experience with the effect of nicotine on the electrical and mechanical activities of the small intestine during rhythmic contraction showed that while the characteristic potential variations were abolished the mechanical contractions continued. In the early experiments an excised intestinal strip was immersed in a bath of physiologic saline solution, and the drug added to the bath. Under these conditions, after the characteristic potential variations had disappeared, they did not again return. For several hours the mechanical contractions continued, at first with full vigor and regularity, then gradually diminishing to complete cessation after three or four hours. During all this time there was no indication of recovery of the characteristic potential rhythm.

The normal characteristic potential variations were attributed to a physiologic rhythm within the structure of the intestine, and it was hypothesized that this depended on the intrinsic nervous plexus. It was plausible to interpret the disappearance of the characteristic potential changes after application of nicotine to a selective effect of that drug on the ganglia of Auerbach's plexus. Since the characteristic potential cycle did not reappear, it was reasonable to infer that this effect was a permanently destructive one.

On the basis of further experiments, it has been concluded that this is not so. Rabbits were anesthetized with urethane, and the lumbar and cervical cord pithed. The intestines were exposed, and a portion of the ileum about 3 cm. long was isolated and tied off at both ends. The mesenteric attachment to the segment of intestine was divided to the root without cutting the main vessels supplying the segment. The segment was then allowed to hang from the animal into a vessel containing 100 cc. of physi-

[1] Work done on a grant from the Josiah Macy, Jr., Foundation under the direction of Walter C. Alvarez, Associate Professor of Medicine, The Mayo Foundation, University of Minnesota.

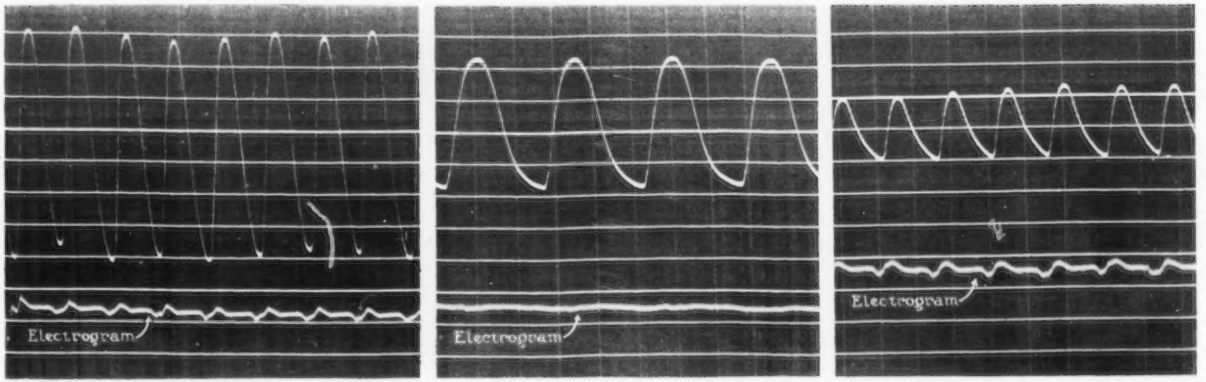

Fig. 1. *a*, Isolated intestinal segment in bath of physiologic saline solution. Blood vessels clamped; usual mechanical and electrical record. *b*, Thirty minutes after nicotine 1 cc., 1:100, was added to the bath. The mechanical rhythm is regular and of large amplitude. The electrogram does not show the typical potential cycle. *c*, Blood vessels unclamped. The electrical alterations return in usual form.

ologic saline solution, and the blood vessels were shut off with rubber covered bull-dog clamps. An electromyographic record was made in the manner described in previous papers. A typical record of the characteristic potential variations in regular relation to the movement of contraction and relaxation was obtained (fig. 1, *a*). Nicotine (1 cc., 1 per cent) was then added to the bath. The usual effects on the electrical and mechanical records that follow immediately after application of nicotine, as previously described, were observed. After thirty minutes the mechanical rhythm was vigorous, regular and slow. The electrical changes were reduced to feeble alterations, showing no characteristic form (fig. 1, *b*). The vessels were now unclamped. Circulation through the intestinal segment was visibly restored, and simultaneously, the typical characteristic potential cycles became evident in the record (fig. 1, *c*). With the vessels clamped, bubbling of oxygen through the bath does not effect restoration of the characteristic potential rhythm, nor does changing of the solution in the bath.

From these experiments it is evident that the disappearance of the characteristic potential variations after contact with nicotine is not the result of an irreparable destructive effect. Probably the presence of the drug in the tissue, possibly the ganglia, prevents the rhythmic physiologic function that is reflected in the rhythmic electrical variations. When the drug is washed out by the circulating fluid this function is restored.

SUMMARY

The characteristic cycles of potential variation that accompany rhythmic contraction of the small intestine, can be abolished by application of nicotine if the circulation of the affected intestine is not active. Restoration of circulation results in recovery of the characteristic potential changes. The abolition cannot therefore be due to a permanent destructive effect on the intrinsic nervous ganglia.

REFERENCE

(1) BERKSON, J. This Journal, 1933, civ, 67.

THE REACTION OF THE RABBIT TO ELECTRIC CURRENTS DIRECTED THROUGH THE HEART

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Received for publication May 29, 1933

This research has been conducted with a view to determining the effects of electric shock when the current is directed through the heart of the rabbit, avoiding the medulla. Thus a fatal issue must be due to some condition other than paralysis of the respiratory centre by the "A. C. block" of Urquhart (1927) and Urquhart and Noble (1929). Death under these circumstances is usually said to be due to ventricular fibrillation.

In the classical experiments of Prevost and Batelli (1899) the observation was made that during the passage of currents of low voltage through the experimental animal, ventricular fibrillation was produced in all mammals; this was transient in the rabbit in most cases, but invariably fatal in the dog. This latter observation has been confirmed by many experimenters; the careful cinematographic and electrocardiographic analysis of the cardiac effects by Wiggers (1930a) has, as well, given us a reliable account of the stages of ventricular fibrillation *per se* in the dog. But the high percentage (63 per cent) of recoveries in man from accidental electric shock from low voltage currents, as reported by Maclachlan (1930), leads one to infer that the human heart is more resistant to such currents than is that of the dog; and the rabbit was investigated in these experiments so that the mechanism of recovery might be understood in an animal in which recovery is common.

Approximately seventy rabbits were shocked through the heart, the electrodes being fastened to the shaved left axilla and right thigh. The axillary electrode was a copper plate, 1.5 cm. square, held in place by a rubber band. For some of the experiments the thigh electrode was a bared copper wire, two inches long, inserted between the thigh muscles and the skin, in others a zinc plate, 2×5.5 cm., tied on the shaved thigh. The effects were studied by kymographic records of respiration, blood pressure and heart rate, by simultaneous electrocardiographic records using lead II, and by direct observation of the exposed heart. The currents used varied from 50 m.a. (34 volts) to 2000 m.a. (1000 volts), 25 cycle A.C. The voltage was varied and measured, along with the amperage and watt hours, by the apparatus installed by the Hydro-Electric Power Commission of Ontario,

and operated by Mr. F. L. Harrison, Testing Engineer. The currents were applied, on the average, for 3.5 seconds.

Most of the animals were anesthetized with ether long enough to be prepared for the experiment; they were then allowed to come out of the anesthetic and shocked. If the first shock was not fatal, succeeding shocks were given without additional anesthesia. A group of animals was shocked with high voltage shocks without anesthesia and without any operative procedure; these animals were allowed to survive and their hearts studied by the electrocardiograph for as long as twelve days afterwards.

The great muscular disturbance which coincides with and follows the electric shock made it almost impossible to get electrocardiograms immediately after the shock. This difficulty was circumvented by giving curare to twelve of the animals to the point when artificial respiration was necessary.

TABLE 1

Fatal first shocks

RABBIT NUMBER	VOLTAGE OF CURRENT	MILLIAMPERAGE OF CURRENT	DURATION OF CURRENT (SECONDS)	DURATION OF RESPIRATION AFTER CURRENT WAS BROKEN
45	38	78	3.1	3 min. 48 sec.
33	45	106	3.0	24 sec.
43	57	120	3.5	23 sec.
74	68	120	3.2	47 sec.
13	72	190	3.7	50 sec.
7	98	260	3.5	14 sec.
3	100	240	3.7	35 sec.
5	140	390	3.6	27 sec.
51	240	450	3.4	Curare

I. Fatal shocks. 1. It is difficult to kill a rabbit by shocking through the heart, missing the medulla. A fatal shock for a healthy rabbit must be below 250 volts (450 m.a.), but even when such low voltages were used, only 9 of 50 rabbits, or 18 per cent succumbed to a first shock (see table 1). Survival from a first shock does not increase the susceptibility to a second, for of the animals which were not killed by a first shock, only 10 per cent were killed by a second; of those which survived the first two shocks, only 11 per cent were killed by a third. Indeed, when a first and second shock of a certain intensity fail to cause death, repetition of this shock is commonly followed by less and less severe cardiac effects, so that a definite tolerance seems to have developed (fig. 1). It was not uncommon to have a rabbit survive seven shocks; one lived after fourteen. After a succession of shocks at a considerable voltage (say 100–300), however, a sudden drop to approximately 50 volts may cause death, and, occasionally, in an animal in low vasomotor condition due to repeated shocks, a current at a voltage

above 250 would produce a fatal fibrillation. These observations hold for successive shocks given at intervals no longer than 30 minutes. A tolerance to electricity developed in the nervous system of the rat by repeated shocks is described by Langworthy and Kouwenhoven (1932).

2. A fatal shock throws the heart first into a ventricular flutter which lasts throughout the period of the shock. The auricles flutter at first, then commonly stop, distended with blood. True ventricular fibillation, con-

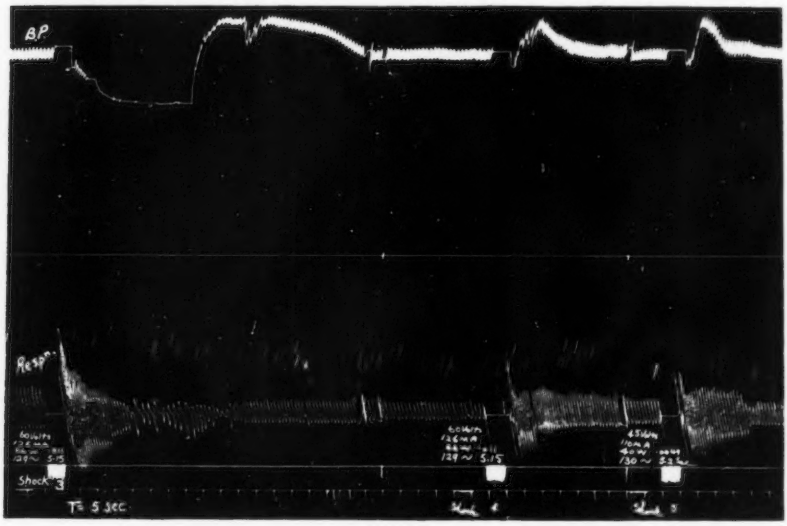

Fig. 1. Rabbit 62. Effect of successive low voltage shocks—tolerance.

The B.P. record was made with a Fick type of manometer; respirations were recorded by a Marey tambour attached to a tracheal cannula. The successive shocks were 126 m.a., 126 m.a., 110 m.a.

After the first shock the B.P. fell promptly due to a ventricular flutter; the waves on the declining B.P. record are due to the violent respirations. With a return to coördination the B.P. rises. A second and third shock have no effect on the heart after the current is broken.

trary to the observations of Prevost and Batelli, was never seen in the rabbit during the *passage of the current* through the heart. In the exposed heart the movements were identical with flutter movements seen during and following the non-fatal shocks, and confirmed by the electrocardiograph. These ventricular flutter-beats are non-expulsive. They continue for a variable time, but less than a minute, after the current is broken; the ventricles then pass gradually into ventricular fibrillation, as described by Wiggers in the dog. Rarely, upon breaking the current, there is a period

of ventricular tachycardia with occasional expulsive beats, lasting as long as twenty seconds; this is followed by flutter and fibrillation. The fibrillation is usually interrupted, in from two to eight minutes by slow coördinate *non-expulsive* ventricular beats. Meantime, the auricles, which had been quiet after they had become distended, usually have picked up a coördinate, feeble, non-expulsive beat long before coördination is restored in the ventricles. Both auricles and the right ventricle are full of blood; there is little in the left ventricle. If stimulants are not given, the ventricles presently slow and stop, but the auricles continue to beat for ten minutes or longer.

The sequence of events leading up to true ventricular fibrillation has been described by Lewis (1925). Following premonitory ventricular extra systoles and tachycardia, there are three stages: first, a "rapid undulation of the ventricular wall," in movements which "probably correspond to flutter of a relatively pure type in the animals," and in which the electrocardiogram

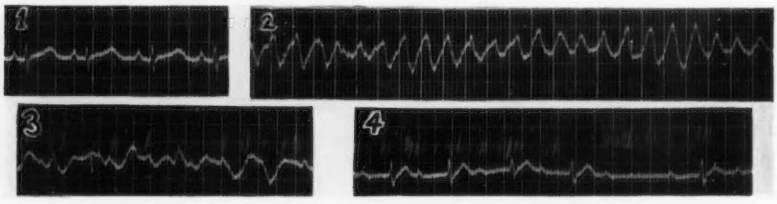

Fig. 2. Fatal shock—rabbit 50. Curare.
1. Normal electrocardiogram before shocking. T = $\frac{1}{5}$ and $\frac{1}{1}$ second.
2. Ventricular flutter, 6 seconds after shock.
3. Ventricular fibrillation, starting 18 seconds after shock.
4. Recovery, non-expulsive beats, long P.R. interval, heart block, 3 minutes after shock.

shows regular rapid waves, with sharp peaks and of even amplitude; second, a transitional stage, characterized by fairly regular, broader, shorter waves with irregular undulations superimposed; and finally, the true fibrillation, in which the waves are completely irregular in size, amplitude and frequency. No expulsion of blood occurs during any of these stages. Wiggers distinguishes four phases in the dog; the first, the stage of "tachysystole," is characterized by regular coördinate peristaltic waves, causing regular electrocardiographic deflections and lasting for less than one second; it is succeeded by a transitional stage of "convulsive incoördination," before passing into the stage of "tremulous incoördination," or true fibrillation.

Because of the uncertainty of inducing fibrillation in the rabbit, it was impractical to take cinematographic records of the heart, and the analysis of the condition was restricted to the electrocardiographic investigation.

The sequence of events as depicted by the electrocardiogram was much as described by Wiggers in the dog: a brief period of tachysystole, lasting, however, for several seconds but less than a minute, then a gradual change to definite incoördination with irregular deflections. This was interrupted in a majority of the animals, by a sudden change to coördinate *non-expulsive* beats, usually atypical, occurring from two to eight minutes after the fatal shock. These were usually from a supraventricular lead, sometimes

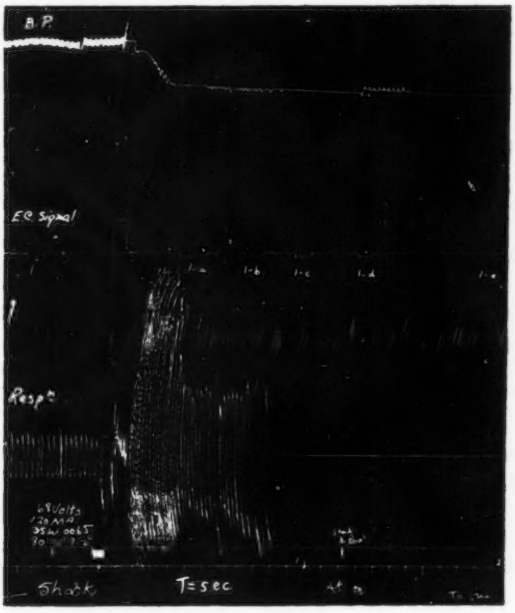

Fig. 3. Rabbit 74. Fatal first shock. 68 v., 120 m.a.

The second line is a signal registering the periods during which electrocardiograms were taken. The first two groups of waves on the B.P. record after the shock are due to the violent respirations; the third group is due to artificial respiration.

from the sinus at first, and at other times nodal,—or even a mixture of the two. The P.R. interval was invariably lengthened, usually to twice the normal, and commonly the early beats, otherwise regular, were succeeded by a progressive heart block (fig. 2).

3. The blood pressure naturally falls to zero immediately after the shock (fig. 3).

4. The immediate effect of the shock on the muscular system is a tonic spasm, with extension of the body and limbs and opisthotonos. There

may be superimposed tremblings. This convulsion lasts throughout the period of the shock, and for as long as eight seconds after; it prevents respiration during this period. Following the release from the tonic spasm there are, provided no previous non-fatal shocks have been given, violent and rapid respirations (fig. 3). These gradually diminish in amplitude and end abruptly after lasting fourteen to fifty seconds, although in one case they continued for more than three minutes. They may be followed, after a brief pause, by one or two gasps. Thus the respiratory failure is late and due to failure of the circulation.

If the animal has received previous non-fatal shocks, its general condition may be quite low and the respirations which follow the fatal shock may have little greater frequency and amplitude than just before the shock, or be subnormal. They are then apt to slowly decline, the last gasp taking place as long as three minutes after the shock. Occasionally, as in three of fourteen animals killed by a second or later shock, the rabbit failed to breathe at all after the fatal shock.

5. A first shock above 450 milliamperes, at 240 volts, 25 cycle, A. C., has not proven fatal when directed through the heart, missing the medulla. The lethal dose was rarely above 300 milliamperes. Thus (table 1) seven of thirty-three rabbits were killed by first shocks ranging from 50 to 260 milliamperes (34 to 100 volts) and two of twenty-nine were killed by shocks ranging from 260 to 2050 milliamperes (102.5–850 volts), viz., at 390 m.a. (140 v.) and 450 m.a. (240 v.). Of the fifty-three animals which survived the first shock forty-eight were shocked a second time, and of these only 4 were killed, by 250 m.a. (150 v.), 340 m.a. (115 v.), 320 m.a. (190 v.), and 480 m.a. (235 v.) respectively.

6. In the group of animals used in these experiments, the heart of the rabbit seemed to be more susceptible to electric shock in May than in the summer months. The susceptibility progressively declined in June, July and August. Thus of thirteen rabbits shocked in May, six or 46 per cent succumbed to a first or second shock below 250 v., i.e., within the fatal range. A similar comparison for June shows a mortality of four out of eighteen or 22 per cent, for July, two of twelve or 16 per cent, and for August one of nine or 11 per cent.

II. Non-fatal shocks. 1. During the period of a shock below 340 volts (790 m.a.) if the heart is exposed in the open chest, the auricles and ventricles are seen to be thrown into a flutter or tachycardia. It may be presumed that currents of equivalent amperage would cause similar effects in the heart in the closed chest. It is difficult to judge whether these beats are expulsive, as commonly the muscular spasm blocks the cannulated carotid artery and the blood-pressure record becomes a straight line. A few records, however, were obtained with apparently little of this blocking; in some there were rapid expulsive beats recorded, with a measurable rise in blood-

pressure, and in others the blood-pressure fell, with no undulations, so that it might be inferred that no blood was expelled. The auricular beats (as seen in the exposed heart) usually cease before the shock is finished, leaving the chambers distended with blood. This condition was never seen in the ventricles during low voltage shocks; they seem to be in a condition of high tonus, not at all well relaxed in the short diastole. It was obviously impractical to endeavour to obtain electrocardiograms during the period of the shock, but the flutter movements looked similar to those which commonly followed the shock for a variable time, and which were recorded by the electrocardiograph.

During shocks at 340 and 350 volts, the exposed auricles stopped, distended, and the distended ventricles quivered rather than fluttered. The fineness of this quivering became more pronounced with currents up to 850 volts (2000 m.a.); it may have been a reflection of a fine tremor of the skeletal muscle of the animal, but, to my eye, seemed to be a property of the ventricles themselves. There was no ejection of blood.

2. The spontaneous recovery of the heart from the flutter movements induced by the shock may be instantaneous, or delayed for periods up to fifty seconds. Only very rarely is it delayed for longer periods, but in one case recovery occurred after three minutes. If there is any measurable delay the blood-pressure falls during that time. When the heart is exposed, the auricles are seen to be quiet and distended. The rapid fluttering movements of the ventricles continue until there is a sudden pause; the ventricles seem to collapse in a noticeable diastole; this is succeeded by slow coördinate expulsive ventricular beats which quickly increase in rate as the auricles presently resume their normal rhythm. With the restoration of ventricular expulsion the blood-pressure rises abruptly.

After the current is broken the occurrence and duration of the ventricular flutter movements can never be predicted and bear no relation to the strength of the current. The reaction seems to vary, rather, with the animal. Thus in rabbit 6, a current of 260 m.a. (105 volts) caused a flutter for fifty seconds after the shock, while in rabbit 4, the flutter movements ceased immediately after the current of 240 m.a. (115 volts) was broken. At voltages up to 100, ventricular flutter persisted for a variable time after the current was broken in 15 of 26 animals, while at voltages above 100 and up to 390, the proportion was about the same, viz., 10 of 20.

Electrocardiograms taken immediately after the current was broken, and while the low B.P. indicated cardiac incoördination, showed rapid regular movements of the fibre, of even amplitude except where they were rhythmically affected by respiratory movements (natural or induced),—quite satisfactory evidence that the cardiac movements corresponded to the ventricular flutter of Lewis, and the tachysystole of Wiggers. These regular movements were registered usually about 600 per minute, but the

rate went as high as 720. (The normal rate of the rabbit's heart under the experimental conditions is from 180–300 per minute.) They are more rapid early in the flutter than just before they end. They end very abruptly and are succeeded after a brief pause (less than one second) by powerful expulsive beats (fig. 4). These beats are slow at first, even while the pressure is low and rising; they are usually of regular sinus origin with normal P.R. interval; there is an occasional non-expulsive ventricular extra systole. There is evidently some sinus depression, and, at times, impaired conduction, from which recovery occurs, however, within two minutes.

It is frequently said that rabbits rarely die of cardiac effects following electric shock, since the ventricular fibrillation induced is usually transient. These experiments lead to the conclusion that they rarely die because ven-

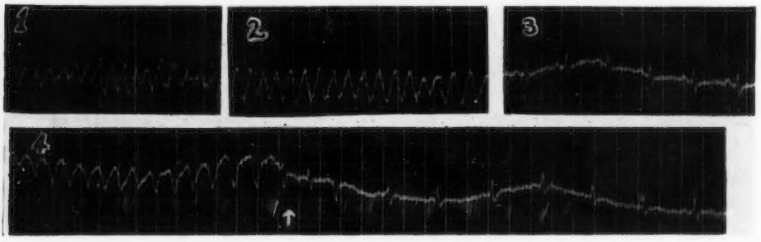

Fig. 4. Non-fatal shock.

1. Rabbit 66. No curare; 10 seconds after onset of ventricular flutter, lasting for 35 seconds.

2. Rabbit 66. No curare; 33 seconds after onset of ventricular flutter, lasting for 35 seconds. The fine waves on the ventricular complexes are mainly due to skeletal muscular tremors.

3. Rabbit 49. Curare—normal before shocking.

4. Rabbit 49. Ventricular flutter (lasting for 4½ sec.) and recovery. B.P. started to rise at arrow.

tricular fibrillation is *very seldom induced* by this method. If ventricular fibrillation is induced it is generally fatal; the condition which usually follows the electric shock is a ventricular flutter, or what Wiggers calls a "tachysystole." If this passes into fibrillation the animal will usually die, but it commonly ends abruptly as described. In only one experiment was recovery spontaneous after a ventricular disorder beyond pure flutter.

3. The effects on the skeletal muscle are similar to those seen with the fatal shocks. During the shock there is profound extension with opisthotonos, and spasm of the diaphragm with a suspension of respiratory movements. Convulsions continue after the current is broken for periods increasing with the voltage of the circuit—up to 30 seconds after voltage over 400. As long as these convulsions are powerful and involve the dia-

phragm, breathing is suspended. Unless flutter of the ventricles has persisted, the blood-pressure rises to a great height during these convulsions. As soon as the diaphragm is released from the spasm, respiration starts. If the spasm has not lasted more than 12 seconds the respirations are extremely violent and deep. They tend at first to maintain or increase the elevated blood-pressure, but diminish rather quickly in depth as the animal becomes quiet and the blood-pressure returns to normal. When the convulsions last for periods of 20 or 30 seconds (as with high voltage shocks), they maintain the blood-pressure at an abnormally high level for this period. The onset of respiration in these cases is slow, the first breaths are feeble; they gradually work up to a rate and frequency above the normal, but rarely to the violence seen after shocks of lower voltage.

It appears, then, that the medulla is not depressed by low voltage shocks, but is greatly excited probably by irradiation and after-discharge. The hyperpnea lasts for minutes after the shock, probably partly due to carbon dioxide resulting from excessive muscular action. In the case of high voltage shocks, however, there seems to be some depression of the medulla even though it is not directly in the path of the shocking current.

III. Survival experiments. Electrocardiograms were taken from four normal rabbits without anesthesia. These were then shocked with high voltage currents—480 v. (770 m.a.), 750 v. (1800 m.a.), 810 v. (1900 m.a.) and 812 v. (2000 m.a.) respectively. All animals survived the shock and were walking about within forty minutes. Electrocardiograms were then taken at intervals that day, and daily thereafter as long as they lived.

One animal (480 v. shock) died within 20 hours; it had diarrhea at the time of shocking and probably died of enteritis. It had no burns on the heart or lungs. Of the other animals, one (810 v.) died on the 6th day, one (750 v.) on the 9th day, and one (812 v.) was killed with ether on the 13th day. All had severe burns of the lungs and some burns of the thigh. The animal which did not die had a severe burn of the left auricle.

The striking observation about these animals was that while they lived and were active no changes were observed in the electrocardiogram, i.e., there was no functional damage demonstrable. Death, in view of the severe changes in the lungs and elsewhere, was judged not to be of cardiac origin.

IV. Histological examination of shocked hearts. Fragmentation of cardiac muscle is often said to be a sequela of electric shock. Paraffin sections of many of the hearts of the rabbits killed with electric shock showed some apparent breaking up of the muscle fibres, but this was not confirmed by frozen sections of the same hearts.

The hearts of nine animals which had died from the effects of a first or later electric shock, and of the four animals which survived the high voltage shocks, were examined for fat by staining sections with Sudan III. If the

hearts survived 45 to 90 minutes after the first shock, it was possible to demonstrate throughout the muscle such a uniform distribution of fine droplets of fat that the whole field had an orange cast. Briefly, the results of this investigation indicate that:

1. Fat appears in heart muscle within 26 minutes of electric shock.
2. It is abundant 45 to 90 minutes after the shock.
3. It is present but in diminished quantity 20 hours after the shock.
4. It disappears within 5 days of the shock.
5. It is more abundant after low voltage than after high voltage shocks. (It will be remembered that there is a tendency for sustained ventricular flutter during and after low voltage shocks that is not seen after high voltage shocks.)

This condition resembles "fatty degeneration," although judging by the quantity present, it is probably due to an unmasking of invisible fat.

Attempts at resuscitation. After a fatal shock artificial respiration was started in practically all cases, with, naturally, no advantage alone. Death was due to ventricular fibrillation which lasted so long that when coördination reappeared the heart was so asphyxiated that the beats were feeble and non-expulsive. The only reasonable methods of resuscitation seemed to be either to attempt to shorten the fibrillation, or to stimulate the heart when coördination was reëstablished.

The only certain methods of stopping ventricular fibrillation produced in dogs by electric currents, are by "counter shock" (Prevost and Batelli, 1899, 1900; Hooker, Kouwenhoven and Langworthy, 1933), and by the introduction of potassium chloride into the coronary arteries via the carotid (Hooker, 1929) or into the ventricular cavities (Wiggers, 1930b) followed by the subsequent introduction of calcium chloride. These methods were not tried, as their usefulness seems limited to the experimental animal. Garrey (1908) was able to stop ventricular fibrillation in the cat by stimulating the vagus nerve. This method was attempted in seven rabbits without success.

Attempts were made to stimulate the heart when coördination reappeared without expulsion. This was determined by direct observation of the exposed heart, and by the electrocardiograph when the chest was intact. The method of Crile and Dolley (1908), i.e., transfusing the coronary arteries through the carotid, with normal saline and adrenalin, was attempted in four rabbits. No animals were resuscitated, although I do not feel that the method was given fair trial, for on two occasions too large a volume of saline was introduced. In a third, ventricular fibrillation was reëstablished. The method was abandoned chiefly, however, because it was found that intravenous injections were more effective, and, being more easily introduced, much more practical.

As soon as the electrocardiograph registered regular coördinate beats,

adrenalin, 2 cc. 1/10,000, was introduced into the jugular vein and the chest compressed rhythmically to massage the heart. Artificial respiration was, of course, carried on continuously. This procedure was carried out on four animals. One was resuscitated, breathed naturally, and forty-seven minutes after the fatal shock was returning to consciousness. Another was resuscitated but after three minutes of good circulatory recovery, suddenly developed ventricular fibrillation again. The other two suffered the accidents of introduction of air into the heart, and rupture of the lungs by too violent chest massage. Unfortunately these experiments were carried on in August, when it was found difficult to kill a rabbit by electric shock, so that although resuscitation was successful in one of the four animals, I do not feel that sufficient attempts were carried out. Introduction of adrenalin before the fibrillation had given way to coördination always resulted in prolongation and intensification of the fibrillation.

SUMMARY

1. It is difficult to kill a rabbit by shocking through the heart. Eighteen per cent of sixty-five rabbits succumbed to a first shock, ten per cent of the remainder to a second shock and eleven per cent to a third.

2. The fatal shocks ranged from 78 to 450 milliamperes at 38 to 240 volts, A. C. 25 cycle. First shocks at 240 to 1000 volts have not proven fatal, and are not followed by any functional damage demonstrable by the electrocardiograph within twelve days.

3. A fatal shock throws the heart first into a ventricular flutter, the auricles fluttering or stopped altogether. The beats are non-expulsive. This condition continues for a variable time after the shock and is followed by ventricular fibrillation. This *may* be followed by coördinate non-expulsive beats appearing 2 to 8 minutes after the shock.

4. Respiratory movements continue, at increased rate and amplitude, for from twenty-five seconds to four minutes after the fatal shock, i.e., after the heart ceases to expel blood. Thus the asphyxia is late and due to failure of the circulation.

5. It is much easier to kill a rabbit in May than during the summer months. The susceptibility declines progressively during June, July and August.

6. If a shock of 240 volts or less is not fatal, the usual effect is a brief ventricular flutter, the beats being non-expulsive. This suddenly changes to regular coördinate expulsive beats. Only rarely does fibrillation occur. In other words, it is rare that the rabbit recovers from ventricular fibrillation, a condition which, however, it is difficult to initiate.

7. If a first shock is not fatal but provokes a long flutter, subsequent shocks of the same voltage within thirty minutes have less and less severe effects, or a tolerance seems to develop.

8. If a rabbit survives a shock, a condition resembling fatty degeneration develops in the heart. The fat appears within twenty-six minutes, is abundant in 45 to 90 minutes and disappears within five days.

9. After a fatal shock, the coördinate non-expulsive beats which appear upon the termination of the fibrillary process may be made expulsive by intravenous injections of adrenalin in sufficient volume to enter the heart, combined with cardiac massage (by compression of the chest wall) and artificial respiration. It is even possible to restore the circulation, make respiration automatic, and have a return of consciousness. The adrenalin must not be given before the fibrillary process has finished, else the ventricular fibrillation will be prolonged. The return of coördination without expulsion can be determined only the electrocardiograph, or by direct observation of the heart.

Acknowledgments. This research, sponsored by the Hydro-Electric Power Commission of Ontario, has been done under the direction of the Committee on Electric Shock of the University of Toronto, two of whose members, Mr. Wills Maclachlan, consulting engineer, and Dr. R. E. Gaby, surgeon, were responsible, with Prof. F. G. Banting, for providing facilities and equipment. I appreciate the loan of an electrocardiograph from Professor C. H. Best, and the assistance of Dr. D. A. Irwin, who made the histological preparations.

REFERENCES

CRILE, G. AND D. H. DOLLEY. 1908. Journ. Exper. Med., x, 782.
GARREY, W. E. 1908. This Journal, xxi, 283.
HOOKER, D. R., W. B. KOUWENHOVEN AND O. LANGWORTHY. 1933. Ibid., ciii, 444.
HOOKER, D. R. 1929. Ibid., xci, 305.
LANGWORTHY, O. AND W. B. KOUWENHOVEN. 1932. Journ. Ind. Hygiene, xiv, 197.
LEWIS, T. 1925. Mechanism and graphic registration of the heart. 3rd ed., Shaw & Sons, London.
MACLACHLAN, W. 1930. Journ. Ind. Hygiene, xii, 291.
PREVOST, J. L. ET F. BATELLI. 1899 Journ. de Physiol. et de Path. gén., 399, 427 et 689.
1900. Ibid., 443.
URQUHART, R. W. I. 1927. Journ. Ind. Hygiene, ix, 140.
URQUHART, R. W. I. AND E. C. NOBLE. 1929. Ibid., xi, 154.
WIGGERS, C. J. 1930a. Amer. Heart Journ., v, 351.
1930b. This Journal, xcii, 223.

A STUDY OF VAGOSPASM

The Action of the Vagus on the Heart During Acute Anoxemia

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Received for publication May 23, 1933

Although several workers have reported observations on the effect of anoxemia on the vagus nerve, it seemed to the authors of this paper that sufficient attention has not been called to the importance of vagospasm. The fact that the vagus nerve has a wide distribution and that anoxemia is produced by many diseases makes this problem an important one.

Mathison (1910) produced anoxemia by allowing the animal to breathe pure nitrogen and paid particular attention to the study of heart block. Eyster and Meek (1914) expressed the view that anoxemia produced a vagospasm. Greene and Gilbert (1922) made an exhaustive study of anoxemia using the rebreather method so that anoxemia was not produced as quickly. In all of this work, however, emphasis was laid on the electrocardiographic studies although the last mentioned authors studied heart rates very carefully.

It seemed then that it would be quite worth while to study vagospasm from a different angle. It was shown by Takeuchi (1925) and Van Liere (1927) and others that the heart grew progressively larger when subjected to rather acute anoxemia. Advantage was taken of this fact to study vagospasm by determining the differences in the size of the heart before and after double vagotomy. The heart was chosen as a criterion in this study as the changes in size could be measured rather accurately.

Methods. The work was done on barbitalized dogs. The trachea was cannulated, a carotid cannula was inserted for blood pressure tracings and the vagi were exposed. No further operative procedures were done. Anoxemia was produced by allowing the animal to breathe pure nitrogen out of a bag which was attached to the tracheal cannula. A steady flow of nitrogen was maintained through the bag. A flutter valve was arranged so that the animal expired to the outside, preventing the accumulation of carbon dioxide. The animal was kept underneath the x-ray machine during the entire experiment and a continuous blood pressure tracing was taken. The distance from the target to the film was kept constant at a

distance of 1 meter. A 5-second exposure was used. A signal magnet marking underneath the blood pressure tracing was arranged so that the exact time and duration of the x-ray exposure could be ascertained. As the exposures were 5 seconds in length, the number of heart beats were expressed in 5 second intervals. A control x-ray picture was taken, anoxemia was then produced and x-ray pictures were taken at the end of $\frac{3}{4}$, 1, $1\frac{1}{4}$ and $1\frac{1}{2}$ minutes. The animal was allowed to return to normal. The vagi were cut. A period of 10 minutes was allowed to elapse and a control picture was taken. Anoxemia was again produced and 4 more exposures were made at the same time intervals as in the control series. The area of the cardiac silhouettes was measured by means of a planimeter.

TABLE 1

	REPRESENTATIVE ANIMAL			AVERAGE OF 10 ANIMALS		
	Cardiac area	Change in cardiac area over normal	Number of heart beats during 5 seconds' x-ray exposure	Cardiac area	Change in cardiac area over normal	Number of heart beats during 5 seconds' x-ray exposure
Before vagotomy						
	sq. in.	*per cent*		*sq. in.*	*per cent*	
Control picture	6.10		12	6.14		12.6
$\frac{3}{4}$ minute anoxemia	6.10	0	11	6.10	−0.65	12.6
1 minute anoxemia	7.10	14.75	11	6.51	6.03	12.2
$1\frac{1}{4}$ minutes anoxemia	8.10	32.78	7	7.13	16.12	9.8
$1\frac{1}{2}$ minutes anoxemia	8.50	39.34	7	7.25	18.07	8.2
After double vagotomy						
Control picture	6.10		13	6.02	−1.9	12.8
$\frac{3}{4}$ minute anoxemia	6.10	0	15	5.97	−2.8	13.3
1 minute anoxemia	6.10	0	17	5.94	−3.3	14.5
$1\frac{1}{4}$ minutes anoxemia	6.50	6.56	18	6.26	1.9	15.0
$1\frac{1}{2}$ minutes anoxemia	6.70	9.84	18	6.68	8.7	12.0

RESULTS. All animals showed cardiac dilatation when subjected for a significant time to acute anoxemia. In some animals the heart shadow was actually smaller at the end of the $\frac{3}{4}$ minute period of anoxemia than the normal. In an occasional animal this held true at the end of 1 minute. The time of appearance of the cardiac dilatation, therefore, varied slightly with individual animals. These observations were found to be true whether or not the vagi were intact. It will be noticed, however, that anoxemia caused the heart to dilate more when the vagi were intact than when the vagi were cut. This is particularly evident in the earlier stages of anoxemia although in the extreme ranges it is less marked or absent.

With the vagi intact, anoxemia caused a progressive decrease in cardiac rate but after double vagotomy it caused a progressive increase except in the extreme ranges where this was not so noticeable.

Atropine may be substituted for double vagotomy with similar but less striking results.

Discussion. It was found that in some instances the differences in cardiac dilatation from anoxemia before and after double vagotomy were not great but the results were all in the same direction and in no case was the opposite found. It is felt that greater differences would have been obtained were it possible to secure an anesthetic which did not partially paralyze the vagus. (We are aware of no such anesthetic.) That is, during the control series the vagi were only partially functioning as the barbital had removed part of their action.

That a correlation between cardiac rate and cardiac size is possible is apparent in the accompanying table. After $1\frac{1}{4}$ minutes of anoxemia the heart beat 7 times during the 5 second x-ray exposure while the vagi were intact, but 18 times during the corresponding interval after the vagi were cut. The cardiac size during the same intervals varied inversely, that is, 8.10 and 6.50 square inches respectively. The mechanism may involve at least two possibilities. First, after double vagotomy any action of the vagi, of course, is removed so that there is no possibility of anoxemia producing vagospasm. Second, as Greene (1926) has pointed out, after the vagi are eliminated, oxygen want may stimulate the cardiac accelerators. Although our results confirm Greene's work as regards heart rate, the exact mechanism requires further study. Furthermore our findings do not conflict with those reported by Schneider and Truesdell (1924) as they did not use such severe degrees of anoxemia as was used in these experiments.

The differences in heart rate undoubtedly could easily account for the differences in the size of the heart as shown by measuring the cardiac silhouette, for Meek (1924) has pointed out that everything else being equal a fast beating heart will cast a smaller shadow than a slow beating one. That the rate is of prime importance is further substantiated by the fact that those animals which showed greater increases in cardiac rate after the vagi were cut showed smaller cardiac silhouettes during the anoxemia period.

It would appear then that anoxemia causes a definite vagospasm, which is not only manifested in changes in the electrocardiographic curve and heart rate but also in actual physical changes as shown by differences in the size of the heart. The vagus stimulation most probably occurs in the medulla. It is not unreasonable to suppose that the vagi may act on other organs in like manner and owing to their wide distribution this would be an important consideration. At the present time work is being carried out on the effect of vagospasm on the pylorus.

SUMMARY

Anoxemia causes less cardiac dilatation concurrently with less cardiac slowing after double vagotomy.

Vagospasm from anoxemia is confirmed by the methods of these experiments.

The greater cardiac dilatation with intact vagi is doubtless caused by the more marked cardiac slowing. Those animals showing greater differences in cardiac rate before and after double vagotomy show greater differences in cardiac dilatation.

Owing to the wide distribution of the vagus nerve it is very probable that other organs besides the heart are influenced by vagospasm. Work is in progress on the effect of vagospasm on the pylorus.

REFERENCES

(1) EYSTER, J. A. E. AND W. J. MEEK. Heart, 1914, cxix, 227.
(2) GREENE, C. W. AND N. C. GILBERT. This Journal, 1922, lx, 155.
(3) GREENE, C. W. The Med. Herald and Physiotherapist, 1926, Kansas City, Mo.
(4) MATHISON. Heart, 1910, ii, 54; Journ. Physiol., 1910, xli, 416.
(5) MEEK, W. J. This Journal, 1924, lxx, 385.
(6) SCHNEIDER, E. C. AND D. TRUESDELL. This Journal, 1924, lxxi, 90.
(7) TAKEUCHI, K. Journ. Physiol., 1925, lx, 208.
(8) VAN LIERE, E. J. This Journal, 1927, lxxxii, 727.

UTERINE BLEEDING OF MONKEYS IN RELATION TO NEURAL AND VASCULAR PROCESSES

I. Spinal Transection and Menstruation

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Received for publication May 26, 1933

The endocrinological approach for investigations of the menstrual cycle has proved so successful that in recent years it has been almost the only one used in the consideration of the mechanisms of reproduction in Primates. Experiment has shown that the injection of the follicular hormone into spayed rhesus macaques (Allen, 1927) and mangabeys (van Wagenen, 1932) is followed, after an interval of some days, by bleeding from the uterus. Similar bleeding ensues in normal rhesus monkeys after the cessation of injections of extracts of the anterior lobe of the hypophysis (Hartman, Firor, and Geiling, 1930; Saiki, 1932). It has also been shown that the experimental menstruation produced in these two ways can be delayed by the administration of progestin (the administration of which is begun as soon as treatment with the anterior lobe preparation or the follicular hormone is ended), and that bleeding will occur a few days after the progestin is stopped (Smith and Engle, 1932). The evidence of endocrine physiology thus suggests that menstruation is the result of the withdrawal of some hormonal stimulus that had been exercising its effect upon the uterine mucosa. The uterine bleeding that follows either bilateral or unilateral ovariectomy is on this hypothesis regarded as being due, not to the removal of the ovaries as such, but to the withdrawal of ovarian secretions.

It is not only the degenerative phase of the endometrial cycle that is controlled by the ovarian hormones, for it has been shown (Allen, 1928; Hisaw, Fevold and Meyer, 1930; and Smith and Engle, 1932) that the same secretions are responsible for the waves of growth in the uterine mucosa. There thus seems to be adequate foundation for the view that the activity of the glandular parts of the reproductive organs is independent of nervous control. Some of Markee's (1932) transplantation experiments support this view. Using rabbits and guinea pigs as experimental subjects, he failed to produce any change in the vascular rhythm of ocular transplants of uterine mucosa, either by cutting or by stimulating the cervical

sympathetic chain. The hypothesis of an endometrial cycle free from nervous influence is however countered by the experiments of Buchheim and Zaleski (1930). Using rabbits, these investigators successfully transplanted fragments of a uterine horn in the subcutaneous tissues of the ear. The transplants, it was found, undergo histological changes similar to those that occur in the control uterine horn after corpora lutea have formed in the ovaries following coitus with vasectomized males. It was also discovered that the changes in the transplants can be greatly intensified if cervical sympathectomy is performed previous to the mating, and the intensification, it is believed, results from an increase in the vascularity of the transplant. This suggests a synergism of hormonal and neuro-vascular processes in the endometrial cycle. The possibility of nervous or neuro-vascular processes influencing the endometrial cycle is given a further measure of support by the results of some experiments, previously reported in abstract (van Wagenen, 1932; van Wagenen and Kennard, 1933) and here described in detail, on the uterine effects of spinal transection.

PROTOCOLS OF EXPERIMENTS. Eight adult and healthy animals were used in the experiments reported below, six being rhesus monkeys (*Macaca mulatta* = *Macacus rhesus* = *Pithecus rhesus*)[1] and two, sooty mangabeys (*Cercocebus fuliginosus* = *Cercocebus aethiops*). All were running normal menstrual cycles.

M. 5, Sooty mangabey (*Cercocebus fuliginosus*). Received in the laboratory June 22, 1931. No bleeding from the uterus was recorded before September; the sexual skin, however, showed cyclical changes, being at its greatest height July 2–7; July 22–30; September 5–15 and October 19–25. Menstrual bleedings, demarcating a cycle of 30 days, were recorded on September 17–23, and October 26–31. The spinal cord was transected on November 2, on the eighth day of the succeeding cycle.

Operation:[2] Body weight, 5,350 grams. 0.8 cc. nembutal per kilogram body-weight was given intraperitoneally, supplemented with ether to gain surgical anesthesia. Cord exposed by laminectomy at about the level of the 10th thoracic vertebra, and divided with scissors.

Post-operative notes: Bleeding from the vagina, lasting 6 days, began on the 4th day after transection. Intravaginal examination, which was made on all the animals used in these experiments, showed the blood to be uterine in origin. The sexual-skin swelling decreased in size during the four day latent interval, and when the post-operative bleeding began was well below the usual resting level characteristic of this

[1] The nomenclature used throughout this paper is that of Flower, 1929.

[2] I am indebted to Dr. Margaret Kennard, to whose surgical skill the successful outcome of the experiments reported in this paper is mainly due, and to Dr. S. Zuckerman for his co-operation in the histological studies connected with this report. Through the kindness of Dr. J. F. Fulton the operations were performed in the Physiology Department of Yale University. To him I also owe thanks for placing two mangabeys at my disposal. The theelin used in the experiments was supplied by the Parke Davis Company, through the courtesy of Dr. Oliver Kamm.

animal at menstruation; the skin continued to decrease until it was down to the level of the callosities. The animal was found dead in the cage on November 28. If the first day of the post-operative bleeding is regarded as the beginning of a new cycle, it died on the 23rd day of the first post-operative cycle.

Notes at autopsy: Death was found to be due to pneumonia. Hind limbs somewhat wasted and contracted. The sexual skin was composed of loose folds around the anus and clitoris, similar in appearance to the sexual skin of a castrate animal. The level of transection was verified as T 10.

Right ovary: Size after fixation 8.0 x 7.0 x 4.0 mm. This ovary contains several follicles between 0.5 and 1.0 mm. in diameter. Toward the medial pole is a large well defined corpus luteum which extends at one point to the surface. Its trabecular structure is still visible, and traces of its initial lobulation are to be seen.

Left ovary: Size after fixation 8.6 x 5.5 x 3.5 mm. It contains small atretic follicles, many of which show hyaline changes. Toward the lateral pole are the remains of an old corpus luteum which approaches the surface of the ovary, and which still shows signs of an antrum. The appearance of its cells, however, suggests that it is older than the corpus luteum of the right ovary.

Uterus: Very little post-mortem change has occurred. The endometrium is low, and in the body of the uterus the straight, narrow glands, which are few in number, do not extend more deeply than one millimeter. The line of demarcation between endometrium and muscle is regular. The glandular epithelium is taller than that of the surface, where many of the cells are almost cuboidal in shape and possess large, round, vesicular nuclei. The nuclei of the gland cells are large oval bodies which do not quite reach the free borders of the cells. The glands are mostly empty, and there is very little secretion in the lumen of the uterus, which, however, contains some débris consisting mainly of what seem to be red blood cells. The stromal cells are tightly packed in all parts of the mucosa, but more especially beneath the surface epithelium and the basement membranes of the glands. The blood vessels of the deeper part of the mucosa are dilated, and in some places the vessels are dilated in all the endometrial zones. Some vascular "lakes" can be seen beneath the surface epithelium which, however, is in no place distorted by their presence.

M. 9, Sooty mangabey.

Weight on arrival, 5,000 grams. Two successive cycles, 30 and 28 days, observed before the operation. Spinal cord was transected on January 14, 1932, the twelfth day of the third cycle. The sexual-skin swelling was not at its height, but was still increasing in the manner characteristic of the follicular phase of the cycle.

Operation: Anesthesia obtained with nembutal. The cord was cut at the level of the eighth thoracic vertebra.

Post-operative notes: On the second day the sexual-skin swelling was less turgid, and on the following day began to decrease in size, the surface becoming wrinkled. Bleeding from the uterus began on the sixth day and lasted for four days. On the twentieth day of the first post-operative cycle (which is estimated from the beginning of the post-operative bleeding) a course of theelin injections was begun, in order to discover whether genital tissues below the level of the lesion could still respond to this hormone, if present; 1200 r.u. were injected over a period of eleven days. The sexual skin increased in size, but did not reach the maximum observed before the transection. Bleeding from the uterus began six days after the cessation of the course of injections. The animal was sacrificed on the second day of this post-theelin bleeding.

Notes at autopsy: Body weight, 4,035 grams. A generalized tuberculous infection was found. Weight of right ovary, 0.142 gram; left ovary, 0.129 gram. Blood in the vaginal lavage before killing: the uterus was found to be full of blood. The transec-

tion level was verified as T 8. The cord showed softening for about 1 cc. on either side of the cut.

Histological notes. Right ovary: Size after fixation 7.7 x 5.5 x 4.0 mm. There are but few macroscopically visible follicles which exceed 0.5 mm. The outstanding exception is a single cystic follicle in the hilum of the ovary which measures 1.5 mm. Many other follicles are in process of atresia, some showing hyaline changes in their walls. There are the remains of two corpora lutea. The more recent of these is situated near the hilum of the ovary and is well demarcated from the surrounding ovarian tissue. Its trabecular framework is still plain. This corpus has no antrum, and projecting from it is a conical expansion which almost reaches the surface of the ovary. The second corpus is much smaller and older.

Left ovary: Size after fixation 8.0 x 5.0 x 4.0 mm. Few of the follicles visible to the naked eye approach 0.5 mm. in diameter. There are many small atretic follicles, of which a large number show hyaline changes. Near the medial pole is a large, fairly-well encapsulated corpus luteum which projects to the surface of the ovary. A smaller and older corpus is to be seen in a superficial position near the lateral pole.

Uterus: The endometrium is deeper than in M. 5, in places being as much as 1.4 mm. thick. Typically the glands are long narrow tubules devoid of secretion. The epithelial lining of both the central cavity and the glands is broken and irregular, and mostly consists of tall columnar cells with irregularly disposed nuclei. The stromal cells are tightly packed, somewhat swollen and irregular in shape. The line of junction between the myometrium and endometrium is regular.

M.m. 21, Rhesus monkey (*Macaca mulatta*).

Received November 21, 1931. Body weight, 5,025 grams. Menstrual cycles of 26 and 22 days observed before the operation, which took place on the 22nd day of the next cycle, February 24, 1932.

Operation: Body weight, 4,980 grams. Four cubic centimeters of nembutal were given intraperitoneally, and ether was administered during the operation. The spinal cord was exposed and cut at a level estimated to be the twelfth thoracic.

Post-operative notes: Bleeding from the uterus, which lasted for five days, began on the third day following the operation. A few red blood corpuscles were seen in the vaginal lavage on the sixth day of the induced cycle, and none was found on the following day. The animal remained well, and bled again on April 1 and on April 29, the dates of bleeding demarcating two post-operative cycles of 35 and 27 days respectively. She was killed on May 24, the fifteenth day of the third cycle.

Notes at autopsy: Body weight, 3,555 grams. Pronounced wasting of musculature of abdomen and lower limbs, with ulceration about the callosities and malleoli. Purulent ulceration of cervix. Weight of right ovary, 0.136 gram; left, 0.107 gram. The level of transection verified as the twelfth thoracic segment.

Histological notes. Right ovary: Size after fixation 7.0 x 6.0 x 3.5 mm. No follicle in the right ovary is more than 1 mm. in diameter; there are about twelve follicles between 0.5 mm. and 1 mm. The many atretic follicles present are not confined to any particular region of the ovary, although they are best defined towards the periphery. Most of the larger follicles seem fairly normal, the process of atresia being evident mainly in the smaller follicles. Many of the atretic follicles are simple fibrous bodies showing hyaline changes, some containing the remains of the degenerated ovum and others having no antrum. The single corpus luteum that is present is not well demarcated from the ovarian tissue, and is pressed in upon by both blood vessels and nearby follicles.

Left ovary: Size after fixation 7.0 x 5.0 x 3.0 mm. There are eight follicles, the largest of which measures 1 mm. in diameter. Towards the lateral pole is a corpus

luteum, much smaller than that in the right ovary and with less distinct cells. The general appearance of the left ovary is much like that of the right.

Uterus: The endometrium, about 1.5 mm. in depth, is of fairly normal interval type. The glands are few in number, and are typical straight tubules containing but little secretion. The glandular columnar epithelium is higher than that lining the cavum uteri. The nuclei of the glandular cells are long oval bodies confined to the deeper two-thirds of the cell. Those of the surface epithelium are somewhat rounder and relatively larger. The stromal cells are tightly packed, and the majority are spindle shaped. They are not swollen as they are in M. 5. The endometrial vessels do not show up well, and the line of junction of endometrium and myometrium, though regular, is somewhat indistinct.

M.m. 22, Rhesus monkey.

Received January 2, 1932. Body weight, 4,015 grams. Menstruation began January 3, and recurred on February 9, indicating a cycle of 37 days. The cord was cut on February 23, on the fifteenth day of the second observed cycle.

Operation: Body weight, 4,105 grams. Anesthesia obtained with 0.8 cc. of nembutal per kilogram body weight. The cord was transected at the level of the eighth thoracic nerve.

Post-operative notes: Bleeding from the uterus began after an interval of seven days, and continued for four days. The animal remained in good condition, and after the post-operative bleeding experienced a cycle of 24 days. She was killed on the 31st day of the second cycle.

Notes at autopy: Body weight, 3,315 grams. Ulceration around callosities and ankles. Internal organs appeared healthy. The sexual skin was a light, clear red in color. Right ovary weighed 0.123 gram; left 0.145 gram. Uterus and vagina contained blood. The level of transection was verified as the eighth thoracic segment.

Histological notes. Right ovary: Size after fixation 7.6 x 5.0 x 4.5 mm. No follicle has as great a diameter as 1 mm.; there are about six between 1 mm. and 0.5 mm. The greater number of follicles seem to be in process of atresia, and many are represented only by small hyaline bodies. One large atretic follicle shows a network of fibroblasts extending through the antrum. There are two corpora lutea. The more recent is compact, solid, regular in shape, and its cells are fairly distinct from each other. It is situated toward the lateral pole of the ovary near the hilum, in which also occur the remains of the second corpus. This one, though larger than the first, is irregular in shape and is badly demarcated from the ovarian tissue. It consists of ill-defined, pale-staining cells. Its stigma is still evident on the surface of the ovary as a scarred depression. Embedded in it is a large atretic follicle.

Left ovary: Size after fixation 7.6 x 5.0 x 4 0 mm. The biggest follicle is 1 mm. in diameter. There are about ten other follicles greater than 0.5 mm., most of which, however, are in process of atresia. Many small atretic follicles showing hyaline changes are to be seen in all parts of the ovary. Three corpora lutea are also present. The most recent, which seems younger than either of the two in the right gonad, is situated in the medial half of the gland, and at one point reaches the surface. It is well encapsulated, solid, and fairly regular in shape. Its cells are mostly distinct from each other, and the supporting trabecular structure is plain. The manner in which the collapsing follicle folded can be inferred from the lobulation, and the part of this corpus which represents the old antrum consists of fibrous strands carrying blood vessels (fig. 1). The second corpus luteum lies near the medial pole and also reaches the surface. Within it is embedded an atretic follicle showing hyaline changes in its wall. The third corpus luteum—still older—is a small irregular body to be seen near the lateral pole.

Uterus: No gland extends more deeply than 1.2 mm., and the glands, which are more numerous than in the preceding specimens, are straight tubules with fairly open lumina, most of which are devoid of secretion. A few of the glands are branched in their deeper parts. The surface and glandular epithelium is broken in places, and the columnar cells of the surface are irregular and not so high as those of the glands. A few mitotic figures can be seen in both the surface and glandular epithelium. The nuclei of the glandular cells are long oval bodies regularly disposed towards the base of the cells. Those of the surface epithelium are somewhat more vesicular, and are irregularly placed. In a few areas the surface epithelium appears to be lifted away from the underlying stroma by accumulations of plasma. There are no subepithelial accumulations of blood cells, but in places the somewhat irregular epithelium is invaded by leucocytes. The cavum uteri contains debris, consisting mostly of degenerated red blood cells, leucocytes and stromal cells. The stroma is not very densely packed, and the stromal cells are either irregularly elongated or spindle shaped. Small accumulations of altered blood pigment occur in the stroma. The time of death was probably the beginning of menstruation.

M.m. 23, Rhesus monkey.

Body weight, 5,125 grams. Menstrual cycles of 38, 24, 33 days observed before the animal was operated upon. Operation on May 11, 1932 on tenth day of fourth cycle.

Operation: Body weight, 5,330 grams. Animal anesthetized with nembutal 0.8 cc. per kgm. followed by ether. Cord exposed in region of eighth thoracic vertebra, and divided with scissors.

Post-operative notes: Bleeding from the uterus was observed on the seventh day after the operation, and continued for one day. The animal was sacrificed on the 22nd day of her second post-operative cycle. The first lasted 35 days.

Notes at autopsy: Body weight, 4,255 grams. Weight of right ovary, 0.148 gram; left, 0.161 gram. Level of transection verified as the eighth thoracic segment.

Histological notes. Right ovary: Size after fixation 7.5 x 7.0 x 3.5 mm. There are about ten follicles between 0.5 and 1. mm. in diameter, most of which are in process of atresia. Many of the smaller atretic follicles have undergone hyaline change. Of the two corpora lutea in this ovary, that nearer the medial pole is the larger. Its cells, however, are not as distinct as those of the smaller corpus (fig. 3).

Left ovary: Size after fixation 9.0 x 6.0 x 4.0 mm. Condition of follicles as in the right ovary. There are two corpora lutea, neither of which appears to be as recent as the younger corpus luteum of the opposite gland. The larger partly covers an atretic follicle which has grown into it from the layer of primordial follicles.

Uterus: The endometrium is low, no gland extending more deeply than 0.8 mm. The line of junction of stroma and myometrium is very irregular, and many glands pass through the stromal zone into the muscle layers. In their deeper parts these glands do not possess so high an epithelium as they do within the region of stroma, where their lining cells are equal in height to those of the surface. The nuclei of both glandular and surface epithelium are tall oval bodies which in the glands are disposed more towards the base of the cells than they are in the surface epithelium. The position of the nuclei in the surface cells makes the epithelium of the cavum uteri appear to be separated from its basement membrane by globules of secretion. There is, however, but little secretion in the lumen of the uterus. The stroma consists of tightly packed, deeply staining, polygonal cells. The blood vessels of the endometrium are not dilated. A few accumulations of eosinophil débris can be seen within the stroma.

M.m. 27, Rhesus monkey.

Received January 29, 1932. Body weight, 4,890 grams. Three successive cycles,

of 33 days, 28 days, and 31 days, were observed before the operation, which took place on May 11, the twelfth day of the fourth cycle.

Operation: Body weight, 4,950 grams. Animal anesthetized with nembutal, followed by ether. Cord exposed and transected at about level of eighth thoracic vertebra.

Post-operative notes: Bleeding from the uterus began nine days after the operation and continued for five days. The vaginal lavage did not show red blood cells on the day preceding the active bleeding. The first post-operative cycle lasted thirty-days, and the animal was killed on the twenty-ninth day of the following cycle, since blood counts and fluctuating temperatures suggested the existence of some general infective process.

Notes at autopsy: Body weight, 4,485 grams. Decubitus ulcers on left ischium, on left ilium, on left internal malleolar and on left plantar surfaces. No ulcers on right side of body. Internal organs appeared normal. Right ovary weighed 0.215 gram; left, 0.187 gram. Level of transection verified as the eighth thoracic segment.

Histological notes. Right ovary: Size after fixation 9.0 x 6.0 x 4.0 mm. The two largest follicles in this ovary, about 1.5 and 1.2 mm. respectively, are in process of atresia. There are eight smaller follicles between 0.5 and 1 mm. There are two corpora lutea, the larger measuring 2.2 mm. in length. Like the smaller it is still connected with the surface.

Left ovary: Size after fixation 8.6 x 6.5 x 4.0 mm. There are about twelve follicles between 0.5 and 1.0 mm. in diameter, the two largest being atretic. About a third of the substance of the ovary is taken up by a centrally situated corpus luteum measuring 3 mm. in greatest diameter. It is irregular in shape, but fairly well differentiated from the surrounding tissue. Its trabecular structure is not defined, nor are the luteal cells distinct from each other. The corpus luteum reaches the surface of the ovary at a point where it is penetrated by fibrous tissue originating from the scar of the rupture point (fig. 2).

Uterus: The outstanding characteristic of this specimen is the very attenuated layer of stroma, which in the body of the uterus does not extend more deeply than at most one-sixth the depth of the glands, some of which are 1.2 mm. long. Both the surface and glandular epithelium consist of glandular cells of medium height, the nuclei of which, especially in the more superficial parts of the endometrium, being somewhat swollen and vesicular. In the deeper parts of the glands the nuclei are more darkly staining, are oval in shape and are regularly disposed toward the base of the cells—the greater part of which they fill. Most of the glands are empty. The narrow superficial zone of stroma consists of closely packed, deeply staining small oval cells. Deeper down these cells irregularly mingle with muscle fibres. The endometrium appears to be in an early phase of "menstruation." The vessels in the superficial parts of the mucosa are dilated, and in several places, particularly beneath the surface epithelium, red blood cells have extravasated into the stroma to form "lakes." The surface epithelium appears to be disorganized over some of these lakes, and blood cells, as well as stromal and epithelial elements, are present in the lumen of the uterus.

M.m. 41, Rhesus monkey.

Received May 6, 1932. Body weight, 5,470 grams. This animal was castrated before its spinal cord was divided. The gonadectomy was performed on June 5, on the twenty-fifth day of the monkey's first cycle in the laboratory. A corpus luteum was present in the left ovary, but there were no large follicles in either ovary. Right ovary weighed 0.166 gram; left, 0.192 gram. The vaginal lavage, beginning eight days after the operation and continuing for five days, contained many red blood

cells; external bleeding was not evident. By June 21 the lavage was typical of a castrate animal. The color of the sexual skin faded gradually. The spinal cord was divided on June 26, 21 days after gonadectomy and nine days after the last appearance of red blood cells in the vagina.

Operation: Body weight, 5,225 grams. Anesthesia obtained with nembutal and ether. The spinal cord was cut at the eighth thoracic segment.

Post-operative notes: Vaginal smears were studied daily for three weeks following the operation. A few red blood cells appeared on the 2nd and 21st days, but otherwise the smears continued to resemble those of a castrate animal.

Notes at autopsy: July 21. Small ulcers in region of pubic symphysis and both rumps. Animal very emaciated. Internal organs appeared normal. Sexual skin colorless. Level of transection verified as the eighth thoracic segment.

Histological notes. Uterus: The uterus is in an advanced stage of super-involution. The glands are very few in number, and only with difficulty can the stromal cells be differentiated from the fibrous tissue which appears to be replacing a great part of the muscle of the myometrium. The epithelium of the surface and glands consists of columnar cells of medium height. The lining cells of the glands are higher than those of the cavum uteri, and their nuclei are more regular in shape, and more evenly disposed. The glands are empty, but the uterine lumen contains amorphous eosinophilic débris, in which can be seen degenerating leucocytes, stromal and epithelial cells. Similar débris is found in very large amounts both in the endometrium and in the myometrium, and in places there are relatively enormous deposits of slightly eosinophilic and neutral material, representing what appears to be a mucinous degeneration of the uterine mucosa. The myometrial vessels are very large, thick walled channels which have undergone considerable hyaline change, and their appearance suggests that the animal was parous.

M.m. 43, Rhesus monkey.

Received May 6, 1932. Body weight, 5,350 grams. First menstruation, May 27–29. The cord was transected on June 3, the eighth day of the first observed cycle.

Operation: Weight, 5,595 grams. Anesthesia obtained with nembutal and ether. Spinal cord divided at the eighth thoracic segment.

Post-operative notes: Sexual-skin color faded on the fourth day following the operation, and on the eighth profuse bleeding from the uterus began. Three days later the animal was killed, so as to obtain a specimen of a uterus in the state of bleeding induced by transection.

Notes at autopsy: Weight, 5,340 grams. Animal in good condition. Wasting and contractures not yet evident. Internal organs appeared healthy. Uterus large and congested. Right ovary weighed 0.297 gram; left, 0.310 gram. Level of transection verified as the eighth thoracic segment.

Histological notes: Right ovary: Size after fixation 11.1 x 7.0 x 4.0 mm. This ovary contains many macroscopically visible follicles, none exceeding 1 mm. in diameter. As in the left ovary, there are many small atretic follicles, some showing hyaline changes. Most of these are to be seen in the peripheral part of the ovary. Some are solid bodies, others have an antrum containing the remains of a degenerated ovum. The central vascular area of the ovary is relatively smaller than in ovaries removed from animals killed a longer period after transection. Towards the medial pole are the deeply embedded remains of an old corpus luteum that is not well differentiated from the surrounding tissue.

Left ovary: Size after fixation 10.8 x 7.0 x 4.5 mm. Condition of the follicles much the same as in right ovary. There are two corpora lutea. The first is deeply embedded, and lies towards the lateral pole. It is irregular in shape, being pressed in

upon by developing follicles, and it consists of strands of ill-defined, pale-staining cells. The second corpus luteum is situated towards the medial pole in the neighborhood of the hilum. It is probably more recent than the other, being more sharply separated from the surrounding tissue.

Uterus: This is a "menstruating" uterus. What remains of the endometrium is 1.3 mm. deep, and except for the superficial fourth the mucosa appears normal. Although this is the third day of bleeding, there has been very little regeneration of the lining epithelium, and in almost all places the superficial stroma and the torn mouths of glands are in direct contact with the débris filling the lumen of the uterus. This débris consists of a matrix of blood cells in which are embedded pieces of gland and stroma. The stroma of the undisturbed part of the endometrium, which is evenly separated from the myometrium, consists of tightly packed, somewhat rounded cells. In this part of the endometrium the glands are fairly straight tubules with open empty lumina. The walls of most of the large blood vessels of the myometrium have undergone hyaline, and what also appears to be mucinous, degeneration (fig. 4).

SUMMARY OF EXPERIMENTAL DATA. a. *Post-operative care of animals:* In almost all cases the operation wounds healed quickly and by first intention. In the first two days following the operation the bladder was evacuated by pressure over the lower abdomen. Bladder automaticity was usually established by the third day. The cages were fitted with straw-filled mattresses, which were changed (usually several times a day) whenever soiled. Some animals were more active than others, and continually dragged themselves by their forelimbs around their cages. The amount of activity shown did not, however, bear any relation to the amount of wasting and contractures that occurred, or to the development of decubitus ulcers. All the operated animals lost weight, but in amounts that in no way corresponded with the period they were kept alive after transection. There is no evidence that the tuberculosis of C.f. 9 began during the period of the experiment.

b. *Temperature records:* Axillary and rectal temperature readings were made both before and after the transection operations. In the normal animal, readings in these two parts of the body closely correspond. After the operation there is a slight fall in the axillary temperature and a much greater one in the rectal. The fall is maintained in both as long as the operated animals are healthy. These temperature data are being presented for publication in a separate paper.

c. *The relation between the level of transection and the time of onset of the post-operative uterine bleeding.* The data are too few to allow any definite conclusions on this point. Five of the seven normal animals were transected at the level of the eighth thoracic segment, the mean length of the latent period before uterine bleeding being seven days, and the range five to nine. The two animals whose cords were divided at lower levels, 10th and 12th thoracic segments, bled after three and two days respectively. It is perhaps significant that in the two cases in which the latent period was

of the shortest duration, the level of section was in that region of the cord generally supposed to be concerned with the innervation of the ovaries and uterus.

The available data do not suggest any correlation between the level of section and the duration of the operative bleeding. Neither do they indicate any relation between the period of the cycle at which the operation is performed and the latency or duration of this bleeding.

d. *The length of the operative cycles:* The "operative cycle" is regarded as comprising the days of the normal cycle that have elapsed before the transection is made, together with the days that separate the post-operative bleeding from the day of transection. As table 1 shows, the operative cycles in the series investigated varied from eleven to twenty-three days, eighteen being the mean length. Both the range and the mean are

TABLE 1

ANIMALS	LEVEL OF TRANSECTION	TIME OF OPERATION (DAY OF CYCLE)	DAYS ELAPSING BEFORE INDUCED MENSES APPEARED	LENGTH OF OPERATIVE CYCLE	DURATION OF MENSES	POST-OPERATIVE CYCLES
					days	*days*
C. f. 5	T 10	8th	3	11	6	
C. f. 9	T 8	12th	5	17	4	
M. m. 21	T 12	21st	48 hours	23	5	35
						27
M. m. 22	T 8	15th	7	22	4	24
M. m. 23	T 8	10th	7	17	1	35
M. m. 27	T 8	12th	9	21	5	30
M. m. 41 (castrate)	T 8		No bleeding			
M. m. 43	T 8	8th	7	15	3+	

considerably lower than the corresponding figures for unoperated animals (Corner, 1923, modal length $3\frac{1}{2}$ to $4\frac{1}{2}$ weeks; Allen, 1927, mean 39 days; Hartman, 1932, mean 28 days).

e. *Length of post-operative cycles:* Post-operative cycles ranging from twenty-four to thirty-five days, with a mean length of 30 days, were observed in three of the animals: M.m. 21, M.m. 22, M.m. 27. The six pre-operative cycles that were observed in these animals ranged from 22 to 37 days, with a mean length of 29.5 days.

The reëstablishment of menstrual cycles in spinally transected rhesus monkeys was recorded by Sherrington in 1900. He writes: "In the rhesus monkey I have seen menstruation come on and recur regularly after spinal transection at the twelfth thoracic level."

f. *The condition of the ovaries:* The ovaries of the spinally transected animals all appeared small and shrunken at post mortem. This fact is well brought out by the weights and dimensions that were taken, and which are given in the protocols.

The ovaries of C. f. 5 were not weighed. The mean ovarian weight for the remainder was 0.175 gram, the range for the average weight for each pair of ovaries being 0.122 gram to 0.303 gram. The corresponding figures for a control series of ten pairs of ovaries removed from normal rhesus monkeys are 0.273 gram, 0.179 gram to 0.343 gram. The mean body weight for this control series was 5.634 kgm., with a range of 4.085 kgm. to 8.920 kgm. The corresponding figures for the operated series were 4.164 kgm., 3.315 kgm. to 5.340 kgm., and are much lower than the control series. It should be remembered, however, that the latter represent post-mortem weights, taken after the animals had wasted considerably. At operation the mean body weight of these animals was in the neighborhood of 5 kgm.

None of the ovaries contained follicles bigger than 1.5 mm. diameter, and all showed numerous atretic follicles, many of which had undergone hyaline changes similar to those figured by Zuckerman (1931). There was at least one old corpus luteum in each pair of ovaries. Thus in M.m. 27 there were two old corpora lutea in the right ovary, and a more recent one, figured in plate 1, in the left. Unfortunately special staining methods were not used in the preparation of the ovarian sections, and with the available hematoxylin-eosin slides it is impossible to decide with certainty the approximate age of the younger corpus luteum of M.m. 27, although there can be little doubt that it was formed either during the operative cycle, or in one of the two post-operative cycles. As is noted below, the uterus of M.m. 27 was in the interval condition, and showed no signs of the action of luteal hormones. This cannot, however, be definitely taken to imply that the corpus luteum did not belong to the post-operative cycles, since there is no evidence for any species showing that the presence of a growing corpus luteum is *always* associated with uterine signs of the activity of luteal hormones. Indeed, these signs are never present in the normal cycles of the unmated rat or mouse, in which corpora lutea develop without achieving functional activity (Deansley, 1930). Again, as Bartelmez (1931) has pointed out, the association in man of a corpus luteum with a progravid uterine mucous membrane, though it may be the typical condition, is not an inevitable one.

These histological difficulties are unfortunately not the only ones which prevent a decision about the occurrence or non-occurrence of ovulation in spinally transected monkeys. Even if corpora lutea had not been seen in any of the ovaries of the animals used in the experiments reported in this paper, it would be impossible to declare dogmatically that spinal transection interfered with the processes of ovulation, since, as has been shown by

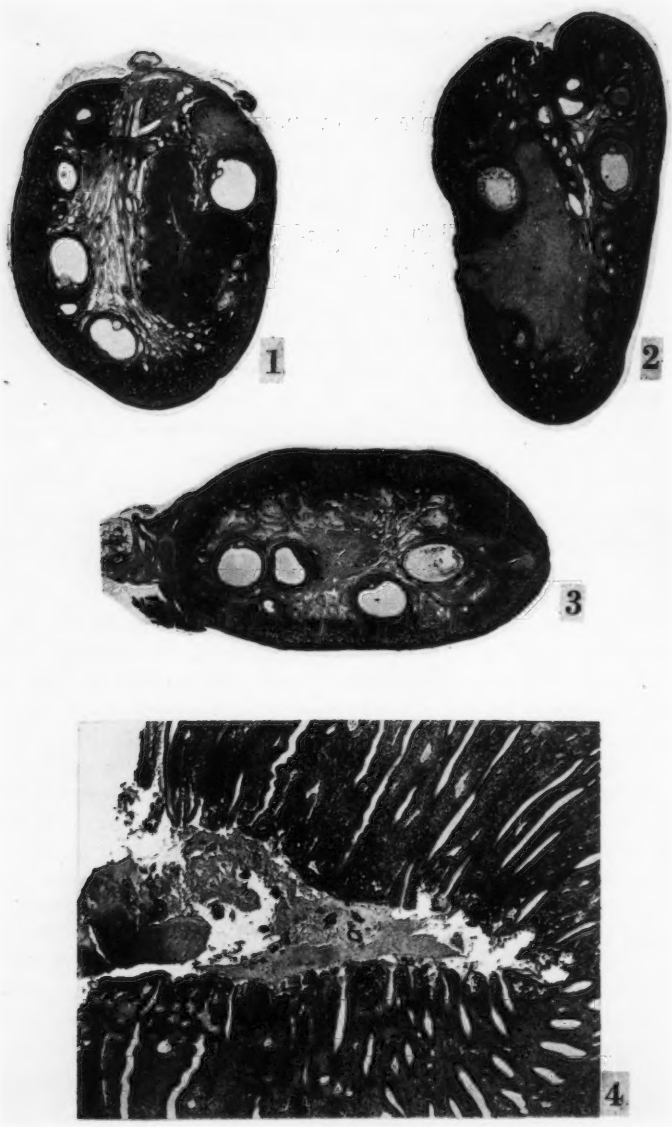

Plate 1. Fig. 1. The left ovary of M. m. 22, showing what appears to be a relatively recent corpus luteum, as well as the remains of an older one near the hilum.

Fig. 2. The left ovary of M. m. 27, showing a large solid corpus luteum. Note the presence of a few atretic follicles with fibrous walls.

Fig. 3. The right ovary of M. m. 23. Many atretic follicles can be seen beneath the peripheral zone, the greater number showing hyaline changes in their walls.

Fig. 4. The uterus of M. m. 43, showing its condition on the third day of bleeding following transection of the cord.

many workers, the animals might have presented just as characteristic a quiescent appearance of their ovaries even if not operated upon.

g. *The condition of the uterus:* The uterus was small in all the operated animals, in none of whom was it actively secreting. The glands were long, straight, and empty as is typical of the "interval" condition. Those uteri that were "menstruating" presented a picture somewhat similar to that of menstruation from an "interval endometrium." Signs of degeneration (atrophy or involution) were present in most of these specimens, and were most conspicuous in the spinally transected castrate animal (M.m. 41). The general histological picture of uterine degeneration resembled that of lactation involution (cf. Zuckerman 1931, plate 1, fig. 5).

h. *The sexual skin:* The sexual skin in both mangabeys had begun to decrease in size before the post-operative bleeding began. Definite sexual-skin changes were not noted in the rhesus monkeys. Since the sexual skin of C. f. 9 proved sensitive to theelin injections, it may be tentatively assumed that the decrease in the two mangabeys was due not so much to some change in the tissues occasioned by the transection, as to a post-operative decrease in the amount of the follicular hormone circulating in the animal's blood.

Discussion. The experiments reported in this paper do not reveal the mechanism of the uterine bleeding that follows spinal transection. The level of transection was in all cases either within or close to the neural segments which are believed to innervate the generative organs, and undoubtedly the primary event in the processes which led to the bleeding was some neural disturbance. With the available data it is, however, impossible to decide whether this disturbance affected the uterus directly, or indirectly through the ovaries, whether its effect was an immediate one upon the tissues of the reproductive organs, or an indirect one through their vascular supply. The single experiment with the spayed female (M.m. 41) which failed to bleed after transection, suggests that the uterine effect is transmitted by way of the ovaries, and in the light of previous knowledge it is conceivable that the transection and consequent neural disturbance affect the supply of ovarian secretions. A diminution in the amount of these secretions might result from some change in the ovarian blood supply, rather than as a direct trophic effect upon the secreting cells of the ovary. Fontaine and Herrman (1932) report that in women, section of the superior hypogastric nerves after about the eighth day of the cycle is followed some 24 to 48 hours later by uterine bleeding, and they suggest that this effect is the result of the intense uterine congestion which follows pelvic sympathectomy. This interpretation goes far beyond the facts already available. Further investigation is essential if the mechanism of this type of uterine bleeding is to be revealed.

SUMMARY

1. The spinal cords of seven normal monkeys (two mangabeys, *Cercocebus fuliginous*, and five Rhesus monkeys, *Macaca mulatta*) were transected at different times in the earlier half of the menstrual cycle. This procedure was followed in all cases by bleeding from the uterus after an interval of from two to nine days.

2. Transection was performed at different levels between the eighth and the twelfth thoracic segment. The latent interval preceding the induced bleeding appears to be shorter the lower the cord is divided.

3. The operative cycle, which is regarded as comprising the number of days from the first day of the last pre-operative bleeding to the beginning of the first post-operative bleeding, is considerably shorter than the mean length of the menstrual cycle in unoperated animals.

4. Following the post-operative bleeding, the normal menstrual rhythm is reëstablished.

5. Both the uterus and the ovaries undergo atrophic changes after the operation. Experiment showed that the sexual skin remains sensitive to theelin.

6. Transection of the spinal cord in a single castrate animal was not followed by uterine bleeding.

REFERENCES

ALLEN, E. 1927. Contrib. to Embryol., Washington, xix, 1.
1928. Amer. Journ. Anat., xlii, 467.

BARTELMEZ, G. W. 1931. Amer. Journ. Obstet. and Gynec., xxi, 623.

BUCHHEIM, W. AND W. ZALESKI. 1930. Compt. rend. Soc. de Biol., civ, 896.

CORNER, G. W. 1923. Contrib. to Embryol., Washington, xv, 73.

DEANSLEY, R. 1930. Proc. Roy. Soc. London, cvi, B, 578.

FLOWER, S. S. 1929. *Vertebrate List.* Vol. 1, Mammals. Zoological Society, London.

FONTAINE, R. AND G. HERRMANN. 1932. Surg., Gynec. and Obstet., liv, 133.

HARTMAN, C. G. 1932. Contrib. to Embryol., Washington, pub. no. 134.

HARTMAN, C. G., M. E. FIROR AND E. M. K. GEILING. 1930. This Journal, xcv, 662.

HISAW, F. L., H. L. FEVOLD AND R. K. MEYER. 1930. Anat. Rec., xlvii, 300.

MARKEE, J. E. 1932. This Journal, c, 32.

SAIKI, S. 1932. This Journal, c, 8.

SHERRINGTON, C. S. 1900. The spinal cord. In *A text-book of physiology*, ed. by E. A. Schafer. Vol. ii, Pentland, Edinburgh.

SMITH, P. E. AND E. ENGLE. 1932. Proc. Soc. Exp. Biol. and Med., xxix, 1225.

VAN WAGENEN, G. 1932. Anat. Rec., lii, 40.

VAN WAGENEN, G. AND M. KENNARD. 1933. Anat. Rec., lv, 39.

ZUCKERMAN, S. 1931. Proc. Zool. Soc., London, Part 2, 593.

THE EFFECT OF ANOXEMIA IN NORMAL DOGS BEFORE AND AFTER DENERVATION OF THE CAROTID SINUSES

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Received for publication May 19, 1933

The hypotheses explaining anoxemic hyperpnea fall into two general classes. One class favors the view that the primary action of the anoxemia is on the respiratory center, the other favors the view that the primary effect is on certain structures in the regions around the carotid sinuses.

There are numerous modifications of the first view. Rosenthal (1882) suggested that the respiratory center is stimulated directly by lack of oxygen while Pflüger (1868) maintained that the center reacts to metabolites circulating in the blood stream which are increased during oxygen want. Hermann (1870) thought that changes in the oxygen content of the blood affect the excitability of the center to carbon dioxide. More recently Gesell (1923) proposed that anoxemia increases the acid metabolism of the respiratory center and the acidity of the center itself is responsible for the ensuing hyperventilation. These views have been summarized in detail by Gesell (1923, 1925). In contrast to these views is that of Heymans, Bouckaert and Dautrebande (1930), who state that the action of anoxemia is through the carotid sinuses. Heymans and his co-workers found that the respiratory response to changes in the carbon dioxide content of the blood, to anoxemia, pH, cyanide and to other respiratory stimulants is diminished or abolished by denervation of the carotid sinus regions. From a histological study of the carotid sinus and of the carotid body, de Castro (1927–28) had suggested previously the possibility that the nerve endings in the adventitia of the sinuses are responsible for the pressure reflexes while those in the carotid body are sensitive to chemical changes in the blood. Bouckaert, Dautrebande and Heymans (1931) have presented experimental evidence that this is the case. Schmidt (1932) repeated and extended the work of Heymans and his associates on the effect of carbon dioxide and oxygen on the sinuses and found that complete denervation of the sinuses destroys the respiratory reaction of the animal to anoxemia. In contrast, the response of the respiratory center to changes in carbon dioxide content of the blood is much greater than that of the carotid sinuses. Schmidt was able to differentiate the pressor mechanism from the chemical mechanism.

He showed that ligation of the occipital arteries at their origin removed the chemical but not the pressor reflexes. Since the occipital artery supplies the carotid body this work gives additional evidence that the carotid body still may be concerned with the chemical reflexes. The work of Heymans and his associates and of Schmidt was done under acute conditions with the animals anesthetized and subjected to severe operative procedures.

The only method of proving whether the primary action of anoxemia is on the respiratory center or on the nerve endings in the carotid sinus regions is to refer this problem to normal animals where the experimental results are not conditioned by anesthesia or acute operations. Accordingly, we have tested the effect of anoxemia on normal dogs before and after carotid sinus denervation, using the term "normal" to indicate that the dogs were not under anesthesia nor acute operations at the time of the experiments.

METHODS. Dogs were trained to breathe through respiratory valves connected to plaster of Paris masks which were constructed and used according to the method described by Marshall (1926). By means of a three-way valve connected to the inspiratory side of the respiratory valve, the dog was subjected to air from the room or commercial nitrogen contained in a Douglas bag. The nitrogen was passed from a tank through water in order to saturate the gas partially with water vapor. For control experiments the Douglas bag was filled with air in order to see if the experimental method had any effect on the respiratory activity. The expiratory side of the valve was connected to a ten liter Bohr meter[1] in order to measure the minute volume of the expired air. The rate and the qualitative changes in amplitude of respiration were obtained by placing a pneumatic pneumograph around the lower portion of the dog's thorax.

After a dog had learned to coöperate, experiments were done in the following manner. The dog was allowed to rest quietly with his nose inserted into a mask When the breathing of the dog had become regular, several determinations of the minute volume of the expired air were made. Then, without interrupting the record, the valve was turned so that the dog inspired nitrogen from the Douglas bag. The assistant on turning the valve pushed a foot-switch connected to an electromagnetic key to indicate on the record the time of breathing nitrogen. This time varied from one-half minute to one minute depending on the length of time the dog submitted to the procedure. If the dog struggled, shorter periods were tried until hyperpnea was obtained unaccompanied by any struggle reaction. This period was terminated by turning the valve so that the animal breathed atmospheric air again. Several determinations of the minute volume of expired air were obtained for the recovery period. Thus this method gave an uninterrupted record of the normal minute rate and volume of respira-

[1] The authors wish to thank Dr. S. W. Britton of the University of Virginia for lending us this meter.

tion for several minutes before breathing nitrogen, the change while breathing nitrogen and recovery from anoxemia. The dog's reaction to carbon dioxide was obtained in a similar manner with mixtures of carbon dioxide and air or carbon dioxide and oxygen in the Douglas bag. For control experiments, the bag was filled with air and the same procedure followed as in the experiments with nitrogen.

After establishing the reaction of the dog to nitrogen, air and carbon dioxide by numerous experiments, the carotid sinuses were denervated. Two types of operative procedure were used. In three dogs the carotid sinus and carotid body were completely removed on both sides. The objection may be made that ligation of the two common carotids and removal of the carotid sinuses may affect the blood flow to the respiratory centers as well as denervate the carotid sinuses. Since the medulla is supplied chiefly from branches coming from the vertebral arteries and since the dogs with the carotid sinuses removed showed no abnormal reactions to their usual environment we do not feel that this is a serious objection. Nevertheless, one denervation experiment was performed without ligation of the carotid arteries. In this dog, the carotid sinuses were denervated by adventitial stripping and phenolization of the region at the bifurcation. Similar results were obtained with both types of operative procedure.

In the excision of the carotid bifurcation, a linear incision 4 to 5 cm. in length was made just below the mastoid process and lateral to the external jugular vein. The underlying muscles were separated and the carotid sheath exposed and opened. The common carotid artery was doubly ligated with silk sutures a few centimeters below the bifurcation. Then the branches beyond the bifurcation were crushed and ligated and the area between the ligatures removed. The fascia of the muscles was approximated with figure-of-eight silk sutures and the skin closed with interrupted subcuticular silk sutures. The carotid bifurcation on the other side was removed in the same manner.

In the second procedure used in dog III, the carotid bifurcation was exposed in the manner described above and denervated by the method used by Gemmill, Overstreet and Hellman (1933) in their study of the effect of clamping the common carotids on pulse and respiratory rates before and after denervation of the carotid sinuses. As much as possible of the adventitial layer of the arteries was stripped away approximately 1 cm. from each side of the bifurcation. The smaller arterial branches in this region were ligated and cut, leaving the internal and the external carotids intact. After the surrounding structures had been protected by gauze packing, this area was painted with 88 per cent phenol followed by 70 per cent alcohol. The operated region was washed with physiological saline and the closure completed. All of the animals recovered uneventfully with the exception of dog II, whose wound on one side opened superficially four days post-

operatively. This wound was treated antiseptically and its secondary closure occurred promptly.

RESULTS. The results are given in table 1 and in figures 1 to 4. In order to have a common basis for all experiments the changes are reported as volume of expired air per minute, although in a number of experiments especially before denervation, the dogs were subjected to nitrogen for only one-half minute periods. If all the dogs studied had been able to withstand the effects of breathing nitrogen for one minute the averages for the results before the operations would have been much higher. Thus the contrast

TABLE 1

Average differences of expired air in liters per minute before and during breathing air, nitrogen or carbon dioxide before and after operating

	WEIGHT	TIME OF BREATHING AIR OR N_2 FROM BAG	AIR				NITROGEN					CARBON DIOXIDE					
			B		A		B		A			B			A		
			Liters per minute	Number of experiments	Liters per minute	Number of experiments	Liters per minute	Number of experiments	Liters per minute	Number of experiments	Number of experiments with gasps	Percentage CO_2	Liters per minute	Number of experiments	Percentage CO_2	Liters per minute	Number of experiments
	kgm.	*minutes*															
Dog I		1	0.3	3	0.3	3	4.5	9	0.5	12	10				4.7	1.2	2
															12.8	4.0	3
Dog II	8.9	½					1.5	9	0.2	8	5	4.7	1.7	3	5.4	1.7	3
		1	0.3	6	−0.1	4			−0.2	2	2						
Dog III	17.3	½					4.0	26	0.0	11	5	5	4.1	1	5	4.6	1
		1	0.0	5	0.0	4			0.0	7	7						
Dog IV	15.0	½					4.4	16	0.0	9	2	5	3.2	2	5	4.0	2
		1	0.1	10	−0.2	3			0.3	2	2						

B, before operation.
A, after operation.

with the results after denervation would have been much greater than those obtained by the one-half minute periods multiplied by two as reported in our table.

A typical result of the change in respiration of a dog before and after denervation of the carotid sinuses while breathing nitrogen is shown in figure 1. Within three respirations after the dog started to breathe from the bag containing nitrogen the respiratory depth increased. This rapid increase is similar to that obtained by Lutz and Schneider (1919–20) in man breathing nitrogen. Then a deep gasp was made followed by another pro-

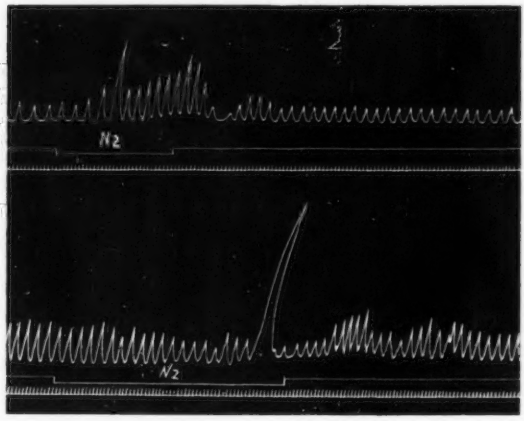

Fig. 1. Dog III. Effect of breathing nitrogen. Upper figure: before denervation. April 5, 1933. Lower figure: after denervation. April 25, 1933. Time in seconds.

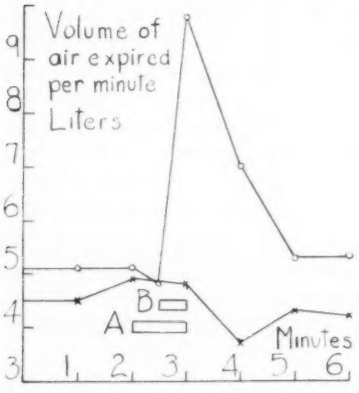

Fig. 2

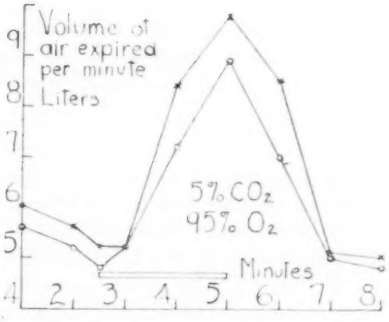

Fig. 3

Fig. 2. Dog III. Effect of breathing nitrogen on the expired air in liters per minute. *O*, before denervation. April 5, 1933. *X*, after denervation. April 25, 1933. *B*, period during which nitrogen was administered for one-half minute before denervation. *A*, period during which nitrogen was administered for one minute after denervation.

Fig. 3. Dog III. Effect of breathing a mixture of 5 per cent CO_2 and 95 per cent O_2 for two and one-half minutes. *O*, before denervation. April 3, 1933. *X*, after denervation. April 26, 1933.

gressive increase in amplitude. Towards the end of the half-minute period there was an increase in rate. After the valve was turned so that the dog breathed atmospheric air again, the depth of respiration dropped quickly to a low level and the breathing became periodic but soon resumed a regular rate and depth.

The results obtained with the same procedure after denervation were in marked contrast. When the valve was turned so that the dog breathed nitrogen there was no initial increase in depth. For the first six respirations the amplitude was practically the same as during the period of breathing

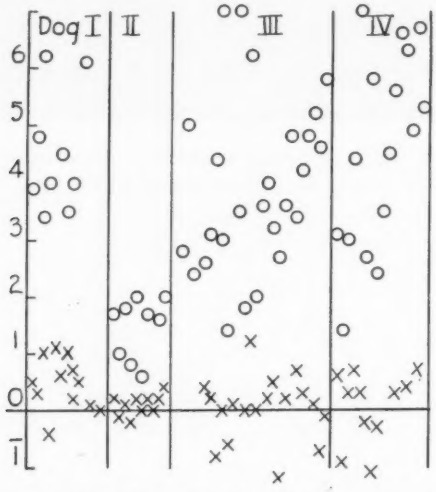

Fig. 4. Differences in volume of expired air in liters per minute before and during breathing nitrogen. Results of 60 experiments before denervation and 51 experiments after denervation in four dogs. In dogs I, II and IV the cartoid sinuses were removed, in dog III, the cartoid sinuses were denervated. *O*, before denervation. *X*, after denervation.

room air. Then the depth decreased and became irregular. A tremendous gasp supervened followed by very shallow breathing. On turning the valve so that the dog breathed room air again, the breathing was periodic for a short time. Finally, the respiratory rate and depth became regular.

A graphic record of the changes in minute volume for these two experiments is given in figure 2. Before operation the minute volume increased from 4.8 liters to 9.8 liters per minute while the dog was subjected to the nitrogen. The return to normal was complete in two minutes. After operation the results were entirely different. There was a slight depression during the administration of the nitrogen which was followed by a greater

decrease of the minute volume after readmitting room air. This secondary decrease occurred in a large number of our experiments, especially after breathing the nitrogen for one minute.

The results of all our experiments are given in graphic form in figure 4 and the averages of the results in table 1. These results were obtained by subtracting the volume of air expired during the minute before breathing nitrogen from the volume of air expired during the minute of breathing nitrogen or the half-minute volume multiplied by two. There was considerable variation in the reaction of the dogs before operation. Not only did the dogs differ among themselves but an individual dog differed from experiment to experiment. This may be due to different degrees of mixing in the lungs in different experiments. The effect of breathing nitrogen on the minute volume of dog II was much less than in the other dogs due perhaps to its smaller size. However, the results presented in the table and in the figures show clearly that the hyperpnea of normal dogs produced by breathing nitrogen is abolished by denervation of the carotid sinuses. After denervation, a slight increase in the minute volume was observed in the majority of the experiments in spite of the depressor action of the nitrogen. In some cases this was due to the large amount of air expired during the solitary gasp. Out of twenty-three experiments after denervation, in which nitrogen was breathed for one minute, the gasp occurred in twenty-one experiments. Out of the twenty-eight experiments during which nitrogen was breathed for one-half minute gasping occurred in twelve experiments. The gasp came either at the end of the period of nitrogen administration or soon after the dog was allowed to breathe room air again. Occasionally a dog struggled after denervation while breathing nitrogen but the struggling was not preceded by an increase in the depth of breathing.

In contrast to the absence of respiratory reaction to anoxemia following denervation, is the ability of the operated dogs to react to carbon dioxide. Mixtures of various amounts of carbon dioxide with air or oxygen were administered to the dogs for varying intervals before and after denervation. The results are given in table 1. These results were obtained by subtracting the volume of air expired for one minute before giving the carbon dioxide mixtures, from the volume of air expired during the last minute of breathing the carbon dioxide mixture from the bag. A graphic record of the minute volume changes in dog III while breathing a mixture of 5 per cent carbon dioxide and 95 per cent oxygen before and after denervation is given in figure 3. The reaction is practically the same in both cases.

When the bag contained air, practically no change was observed in the minute volume of respiration or in the graphic records of respiratory rate and depth. This illustrates further that the experimental procedure had no effect on the respiration of these animals.

Discussion. Hitherto the study of the mechanism by which man and normal animals respond to low oxygen percentages has been complicated because of the failure to recognize the difference in the effect of anoxemia on the carotid sinuses and on the higher centers. Our results show clearly that the primary effect of anoxemia in normal dogs is on the nerve endings of the carotid sinus regions. We cannot offer any explanation of the actual nature of the effect on the nerve-endings producing this reaction. It is interesting that the lack of a chemical substance stimulates nerve endings into action. All of the theories which have been suggested for the supposed stimulatory action of anoxemia on the respiratory centers in the brain may be applied to the carotid sinus regions, except the theory that lack of oxygen increases the sensitivity of the respiratory center to carbon dioxide. Since the primary sites of action to lack of oxygen and to changes of carbon dioxide content are anatomically and functionally separate, this theory no longer has any value. The actual mechanism of stimulation has not been explained but it has been removed to another position.

For the first time the true effect of anoxemia on the higher respiratory centers has been obtained in dogs unaffected by anesthesia or acute operative procedure. This effect is generally a depressor one, the only excitatory change being a gasp. This gasp may be the result of a stimulation to the primitive gasping center postulated by Lumsden (1923). The gasp occurred in nearly all of our experiments after denervation where the nitrogen was administered for one minute. It occurred towards the end of the period of anoxemia or soon after the valve was turned back to atmospheric air. In addition, our results show that the carotid sinuses are not necessary for the control of breathing under the usual environmental conditions. They are necessary, however, for the respiratory reaction of dogs to oxygen-want.

CONCLUSIONS

A separation has been obtained between the respiratory responses to breathing nitrogen and carbon dioxide in four normal dogs unaffected by anesthesia or acute operative procedures.

By denervation of the carotid sinus regions the hyperpnea normally produced by breathing nitrogen was abolished. Thus the primary effect of anoxemia on the higher centers was obtained. This was generally a depression, the only excitatory change observed being a solitary gasp.

The respiratory reaction of these dogs to carbon dioxide was not abolished by denervation of the carotid sinus regions.

The authors wish to thank Mr. J. Whitridge, Jr., for his aid in these experiments.

REFERENCES

BOUCKAERT, J. J., L. DAUTREBANDE AND C. HEYMANS. 1931. Ann. de Physiol. et de Physicochim. Biol., vii, 207.

DE CASTRO, F. 1927–28 Trav. Labor. Rech. Biol. Univ. Madrid, xxv, 331.

GEMMILL, C. L., E. W. OVERSTREET AND L. M. HELLMAN. 1933. This Journal, civ, 433.

GESELL, R. 1923. This Journal, lxvi, 5.
1925. Physiol. Reviews, v, 551.

HERMANN, L. AND T. ESCHER. 1870. Pflüger's Arch., iii, 3.

HEYMANS, C., J. J. BOUCKAERT AND L. DAUTREBANDE. 1930. Arch. Int. de Pharmacody. et de Thérap., xxxix, 400.

LUMSDEN, T. 1923. Journ. Physiol., lviii, 81.

LUTZ, B. R. AND E. C. SCHNEIDER 1919–20. This Journal, l, 327.

MARSHALL, E. K., JR. 1926. This Journal, lxxviii, 459.

PFLÜGER, E. 1868. Pflüger's Arch., i, 61.

ROSENTHAL, J. 1882. Hermann's Handb. d. Physiol., iv, 165.

SCHMIDT, C. F. 1932. This Journal, cii, 94, 119.